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A Study of Mosquito Behavior[1]

An Experimental Laboratory Study of the Sexual Behavior of Aedes aegypti (Linnaeus)[2]

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1 The work described in this paper was done under a contract between the Quartermaster Corps, Army of the United States, and The Ohio State University Research Foundation.

2 A thesis submitted in partial fulfillment for the degree of Doctor of Philosophy in the Graduate School of The Ohio State University, Columbus, Ohio.

Introduction

Many of the observations regarding mosquito mating behavior have dealt with the factors governing swarming, the size and positions of swarms (Howard, Dyar, and Knab, 1912, pp. 120-132; Marshall, 1938, pp. 295-304), and whether or not the species in question would mate in a small space or required a large area for copulation ("stenogamy" and "eurygamy," Roubaud, 1932). Sexual behavior other than the copulatory act itself has been called "epigamic" and recent work on the *Anopheles maculipennis* group has shown that this epigamic behavior apparently is a specific and inheritable character (Bates, 1941). Bates (1941) refers to the special flight of a single male as a "sexual dance" and to a congregation of such dancing males as a "swarm."

It is well known that mosquitoes produce an audible hum during flight which may be so distinctive that some workers claim they are able to identify the species by this sound alone. The literature contains many statements asserting that sound plays an important rôle in the attraction of the sexes of mosquitoes; actually no one has proved that this sound which, at least in part, is produced incidentally during flight, has any biological significance. It has been supposed, though never proved experimentally, that Johnston's organ which is greatly developed in the pedicelli of the male antennae of culicids serves an auditory function.

The positions assumed by mosquitoes during copulation have been recorded by various authors, and Knab (1907b) correlated these attitudes with the extent of development of the female tarsal claws.

The present paper describes various experiments performed to investigate some of the above problems. It was proved that the male of *Aedes aegypti* is attracted to the female by the sound she produces in flight; that the antennae of the male are auditory in function; and that Knab's hypothesis that the position assumed during copulation is dependent upon the degree of development of the female tarsal claws is invalid and was based on the erroneous observation that the female mosquito clings to the male. The last portion of the paper deals with the behavior of male *aegypti* towards mechanically produced sound.

Although most of the experiments were performed on *A. aegypti,* some laboratory observations on other mosquitoes, chiefly *Culex pipiens* L. and *Anopheles quadrimaculatus* Say are included. An attempt has been made to interpret, in the light of recent findings, the many observations on mosquito behavior found in the literature, and also to point out problems which remain to be solved.

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Experimental Methods

Laboratory colonies of *A. aegypti* and *A. quadrimaculatus* were maintained in the University insectary. Later in the work a colony of *Culex pipiens* also

was established. Virgin mosquitoes, when wanted, were obtained by sexing pupae by size (female pupae are noticeably larger than male) and rearing in separate cages. Part of this study deals with the behavior of mosquitoes which had various structures removed by operation, and the method of anesthesia will now be described. For the sake of clarity the various techniques employed are described in the section of the paper with which each is concerned.

Operations were performed on mosquitoes, using iridectomy and microdissecting scissors. Mosquitoes were anesthetized with carbon dioxide after the method (with slight modification) described by Williams (1946). The apparatus is shown in Figure 1. A 3″ diameter Buchner funnel (B) fitted into a wooden plate was substituted for the glass stage of the binocular dissecting microscope. A piece of wire screen, on which rested a small square of filter paper, was fitted into and raised a short distance above the bottom of the funnel. In order to indicate the rate of flow of the gas, the carbon dioxide from a tank (not shown in Fig. 1) was bubbled through a gas washing bottle (C) containing water, before entering the funnel through the rubber tubing (TC). A "Y" tube (Y) was attached to the rubber tube (TA) leading

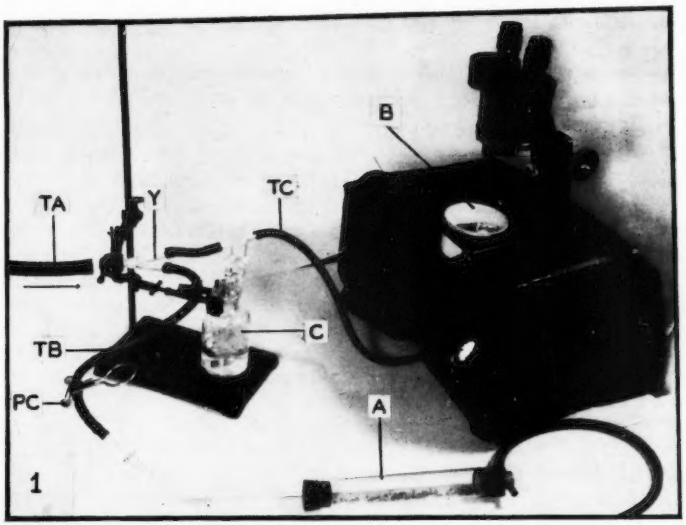


Fig. 1.—Continuous anesthesia apparatus.

Abbreviations: A—aspirator (containing mosquitoes); B—Buchner funnel (with wire screen and anesthetized mosquitoes on square of filter paper); C—gas washing bottle (containing water); PC—pinchcock clamp; TA—rubber tubing leading from tank of CO_2; TB—rubber tube with glass portion of medicine dropper at free end, inserted in one end of aspirator; TC—rubber tube leading into Buchner funnel; Y—Y tube.

Arrow indicates direction of flow of CO_2. (Photograph by W. E. Wagner.)

from the tank of carbon dioxide so that one branch led into the gas washing bottle while the other branch led into a rubber tube (TB) which was kept closed by means of a pinchcock clamp (PC). The glass portion of a medicine dropper was inserted in the end of tubing TB. Mosquitoes removed from cages with an aspirator were anesthetized by inserting the glass end of tubing TB into one end of the aspirator and opening the pinchcock clamp. The anesthetized mosquitoes were placed on the square of filter paper resting on the wire screen in the Buchner funnel. Closing the pinchcock clamp diverts the carbon dioxide through the gas washing bottle and into the funnel so that the mosquitoes surrounded by the gas are under continuous anesthesia. The operations were performed on individual mosquitoes placed on the small piece of filter paper; it was found unnecessary to treat the wounds since clotting apparently occurred rapidly and there was little loss of haemolymph. It is also probable that during the operation the fine scissors brought the cut edges together and helped seal the wound. Males were kept for more than 30 minutes under anesthesia and after recovery showed normal mating behavior. The operations required only a few seconds and many mosquitoes were kept in the funnel at one time; after several individuals were operated upon they were removed and placed in a cage 11″ x 11″ x 15″ for observation and testing.

Sexual Behavior of Mosquitoes

HISTORICAL

Godeheu de Riville (1760) made what was probably one of the earliest recorded observations on the mating behavior of a species of mosquito which Knab believed was *Aedes aegypti*. Knab (1907a) quoted from de Riville's article, "The female, recognized by the length and stoutness of her body, sat in the ordinary position of mosquitoes; she clung to the fabric with her front and middle legs, the two hind legs elevated in a half-circle above her wings. The male, on the contrary, had assumed a different attitude. The smaller size of his body and the necessity of obtaining union with the female, who did not seem inclined to incommode herself, left him apparently less at ease. His two greatly elongated front legs alone held him to the top of the canopy while with the other four legs he grasped the female." Goeldi (1905, p. 74) observed that, indoors, the males of *Stegomyia calopus*[3] tended to gather in groups of fifteen or twenty or more over prominent objects, such as a table or other furniture, while the females flew all about the room. When a female approached one of these groups of males she would be seized by a male which "... unites himself to her from the lower side and permits her to carry him with a slow heavy flight for a few seconds (two or three only), and then again separates himself." Goeldi also noted that this species will copulate in a bottle or breeding cage; that the same male will copulate with many females; and that there are not two distinct swarms, one of males and the other of females, though there is a tendency for the sexes to keep separate.

Howard et al. (1912, p. 275) recorded the observations concerning the mating of *aegypti* made by E. G. Hinds, who noted that "... mating begins

3 *Stegomyia calopus, Aedes calopus, Stegomyia fasciata* = *Aedes aegypti.*

when on the wing. It may, and usually is completed while still flying, but the female frequently alights during the act and before its completion. The complete act requires but the fraction of a minute. According to this observer, females partly or fully fed seem more attractive to males than those which have not fed at all. Copulation sometimes, perhaps usually, occurs before either sex has fed. Males often alight on the dark colored clothing of a person sitting quietly and watch their chance to pounce upon a female coming to feed. The time of the greatest activity of copulation seems to be between 4 and 6 p. m. A male may mate with several females. More than one batch of fertile eggs may be laid without intervening fertilization."

NORMAL MATING BEHAVIOR AND THE ATTITUDES ASSUMED DURING COPULATION

Observations were made on mosquitoes confined in cages 11" x 11" x 15" with cheesecloth sides and glass tops. The floors of the cages were painted white on the inside to facilitate observation. In order to observe the rôle of the legs of both sexes during copulation, a fine thread was glued to the mesonotum of a female and she was suspended from the glass top of a cage. A dissecting microscope with its stage removed was placed on the glass top directly above her. In this way copulation could be observed under high magnification. The following description of the mating behavior of *aegypti* is based on observations of many tethered females and innumerable copulations between free-flying mosquitoes.

In a cage crowded with both sexes, females are seen (1) resting on the sides of the cage surrounded by males which apparently ignore them, (2) copulating with males while resting on the sides of the cage, or (3) flying about the cage usually with one or more males clinging to them. A cage containing about 25 males and 25 females was observed for one-half hour without disturbing the mosquitoes. Very few individuals flew about and few copulations took place. Occasionally a male flying about, briefly touching the sides of the cage, will sometimes touch a female and she takes to flight, whereupon he pursues and seizes her. A female in flight will pass close by a resting male which will take to flight, seize her, and copulate. A pair joined in flight often attracts other males which attempt to seize the same female. As many as 3 or more males will pursue or cling to a single female. The male is the aggressor and after seizing the female in flight attempts to gain a ventral attitude. If several males manage to seize the same female she drops to the floor or lands on the side of the cage. The male which first gains a ventral attitude usually succeeds in copulating. The others still cling to the female (or other males) after she lands, but fly away after unsuccessfully trying to make connection with the female.

If the attitude which a pair has upon landing on the side of the cage is one which does not permit mating (i.e. the male on top), the male will (1) fly away and not return, (2) make some effort to copulate by flexing the abdomen or trying to crawl under the female, or (3) the female takes to flight with the male still clinging to her. In the latter case he reorients himself in flight and copulation is completed in the air, or if the female lands again

copulation generally is successful while the pair is at rest. In most cases the female lands on the side of the cage with the male clinging to her and copulation is completed here rather than in flight. This is in agreement with Hovanitz (1946) who concluded that *aegypti* copulate most frequently after the female comes to rest, while copulation partly in flight and at rest is common and copulation in flight is the least common.

The male and female while copulating are belly-to-belly (face-to-face), with their heads pointing in the same direction (Fig. 36). The front and middle legs of the male are used to seize the female, his hind legs, initially, taking no part in the process. The female is completely passive during the seizing process and does not hold the male to her. When the male seizes the female and gains a ventral attitude, one of his front tarsi seizes her hind femur near its base while his other front tarsus will seize her other hind leg in the same place or somewhat beyond the base of the femur (from observations on seizure of tethered females). At other times, after gaining a face-to-face attitude his front legs grasp other parts of the female's front, middle, or hind legs. Although the female is not always seized exactly in the same manner, the male initially seizes some part of her legs.

The last tarsal segment of the front and middle legs of the male are hollowed on one side (Figs. 17-19) and that part of the female leg which is seized fits into the hollow where it is held by the large tarsal claws of the male. When a female is suspended by a thread, the male clings to her with both his front and middle legs. However, when a free-flying pair come to rest on the side of the cage, the front legs of the male are the ones chiefly used in clinging to his partner; some males bend their front legs backwards to cling to the side of the cage after connection with the female genitalia is accomplished. The middle legs of the male are used to push the abdomen or hind legs of the female upwards until contact with the genitalia is effected; this is particularly true if the male has difficulty in making connection. Once the genitalia are joined, the middle legs push on the tibiae or usually the base of the tarsi of the hind legs of the female and keep them raised in the air; if a female resists, she often uses her hind legs in an attempt to dislodge the male. Sometimes when the pair is in the facing position the tarsal claws of the male's middle legs are hooked over the margins of the female's wings. The hind legs of the male sometimes help, with his middle legs, to push the female abdomen into the proper position for connection. However, when connection is accomplished, the hind legs of the male usually hang downwards or rarely are bent backward and rest on the side of the cage. Once in the proper position, the male flexes his abdomen ventrad until he connects with the female's genitalia; copulation is rapid and the male releases the female and flies away. The angle formed by the abdomen of the male with the nearly vertical abdomen of the female varies from a more or less V-shape to one of about 90 degrees if the male is in a low position. Copulation takes place on the floor of the cage when the female drops with the male directly under her. Occasionally the female in landing on the side of the cage clings to the cloth with only one front leg and copulation takes place with both individuals assuming a sidewise position. Sometimes the male copulates when slightly off to one side of the

female and not directly under her. In a crowded cage males are seen occasionally landing on (or partly on) resting females, but no attempt at copulation is made unless the female takes to flight or vibrates her wings. No males were ever seen to accomplish copulation while astride the female.

Goeldi (1905, Plate 1, Fig. 3a) figured *aegypti* copulating while in flight, in a face-to-face position. The female's legs were drawn extended from her body and were not holding the male to her. However, he showed the male in connection with the female but none of its legs were holding the female. The one male prothoracic tarsal segment shown was extended beyond the female mesonotum. Both pairs of his middle and hind legs were drawn extended beyond the female's abdomen. With none of the male legs seizing the female, the male actually would hang head downward or face in the opposite direction from the female (see Figs. 37, 38), and would not be in the position shown in Goeldi's drawing (cf. Fig. 36).

Lamb (1922) used the terms "pose" and "position" to describe the attitudes assumed by the sexes of different insects during copulation. The pose is the attitude adopted by the individuals just previous to pairing, i.e. at the instant at which the penis is inserted into the vagina. The position is the attitude adopted by the pair in the later stages of copulation. Though the male *aegypti* usually first seizes the female dorsally so that he is uppermost, he must gain a ventral attitude before connection can be made. Assuming that insertion of the penis occurs at the moment the male clasps the female genitalia, there is no change of attitude so that the position ("belly-to-belly" position of Richards, 1927; "facing position" of Hardy, 1944) is the same as the pose. In mosquitoes the belly-to-belly pose ("vertical pose" of Lamb, 1922) is known as the modified linear pose since it is a modification of the linear pose (Lamb, 1922; Richards, 1927). This can be demonstrated by placing a tube saturated with ether fumes over a pair of copulating mosquitoes; if done quickly, the pair will become anesthetized and the male will release his leg hold on the female but they may remain attached by their genitalia. They may then be extended so that the pair is joined tail-to-tail with their heads facing in opposite directions. The male of some species, like *A. quadrimaculatus*, initiates copulation in the modified linear pose and after connection is made releases his leg hold and swings into the linear position (Fig. 38).

Banks (1908) reported that the male *aegypti* flew under the female and clasped her from beneath. Lamb (1922) stated that if Bank's statement ". . . be taken literally, it would appear to involve the male flying upside down at the instant of pairing, which may indeed be just possible, but offers considerable mechanical difficulties. It is possible that, at the first instant of copula, the condition is the same as in *Empis borealis*, namely, an ordinary F. V. P., . . ." (F. V. P. = female vertical pose). Richards (1927, p. 335) commenting on Lamb's statement suggested that the belly-to-belly pose might be accomplished if both insects flew nearly perpendicularly and "They would then have their ventral surfaces apposed without flying upside down." In most instances the male *aegypti* in his initial seizure of the female grasps her dorsally and he is uppermost. Apparently, he rapidly changes to a ventral attitude

while clinging to the flying female. Thus it is not necessary that the male fly upside down when he first seizes the female. Copulation was never accomplished or initiated in the female vertical pose (i.e. with the female above the male and his dorsal surface against her venter) as suggested by Lamb. The pair also may fly perpendicularly as suggested by Richards. The abdomen of the lone female in flight is held nearly horizontally but when she is seized by the male which gains a ventral attitude her abdomen may hang downward so that the abdomens of both male and female are practically perpendicular and the pair fly about briefly in this manner. Hardy (1944) described this as the "vertical poise."

LENGTH OF TIME REQUIRED FOR COPULATION

The length of time the male clings to the female in attempting and successfully completing copulation is variable and depends upon (1) whether there is interference from other males, (2) how quickly the female comes to rest, if copulation is not completed in flight, (3) how quickly the male gains the ventral pose, and (4) whether or not the female resists or submits. Thus the time required for the complete act, from the moment of seizure to the release of the female after connection, usually is lengthened when more than one male pursues and clings to a single female, when the female tends to remain in flight with the male not making connection, when the male does not gain a venter-to-venter attitude quickly, and when the female resists. Conversely, the time is shortened when a male seizes a female with no intervention from other males, when he gains a ventral pose quickly, and when the female does not resist and they complete copulation during flight.

Actual copulation, or the uniting of the male and female genitalia, is a very rapid process. Using a stop-watch, 25 copulations were timed from the moment the male made connection with the female genitalia to the moment he released her, and it was found that the time required varies from 4 to 59 seconds, with an average of about 16 seconds. With repeated copulations a female resists by jerking away her abdomen or pushing the male away with her hind legs. This resistance, though shortening the time the genitalia are joined, usually lengthens the time the male clings to the female, for he makes repeated attempts to connect with her genitalia when she manages to pull her abdomen away before he releases her.

REPEATED COPULATIONS OF MALES

Males will copulate repeatedly with the same or different females, particularly if the cage is agitated whenever the mosquitoes come to rest. This disturbance induces them to fly and the males immediately pursue the females and seize them. If a female takes to flight just as the male leaves her after copulation is completed, the same male may pursue, seize, and copulate with her a second time. One virgin male placed in a cage with 16 virgin females

copulated successfully[4] 30 times and attempted 10 other unsuccessful[4] matings during a 30-minute period. Eleven virgin males copulated successfully 50 times during 1 hour (unsuccessful copulations were not counted) with a single virgin female. In the above cases the cage was agitated to induce flight.

TABLE I.—Results of Mating a Single Male with 17 Virgin Females over a Ten-Day Period.

Age of male (hours)	Number and age of females	Remarks
70 (virgin)	7 (71 hours)	The matings occurred over a one hour period; the first four females that mated had two of their spermathecae filled with sperm; the last three females mated had empty spermathecae.
89 (19 hours later)	3 (81 hours)	The three matings occurred over a five minute period; the first-mated female had sperm only in her middle spermatheca; the other two females were completely negative for sperm.
122 (33 hours later)	4 (age unknown)	The matings occurred over a ten minute period; the first-mated female had sperm in the middle spermatheca only; the other three females were negative for sperm.
168 (46 hours later)	1 (6 days)	Two spermathecae well-filled with sperm.
240 (72 hours later)	2 (8 days)	The first female was lost; the second was negative for sperm.

Banks (1908, p. 246) observed a single male *Stegomyia* copulate with 7 to 8 females. Low (Theobald, 1903, p. 3) noted that after a pair copulate, ". . . the male pursues another female, until it may have fertilized many in a day." Other reports on repeated copulations on the part of male *aegypti* already have been mentioned. However, the mere act of copulation does not necessarily indicate that fecundation has taken place and the following experiment was performed to determine how many females could be impregnated by a single male. One male was allowed to copulate with a number of virgin

4 Copulation was considered successful if the male succeeded in making connection with the female's genitalia and the pair remained joined for a brief period; however, it should be remembered that making connection does not imply that the female has been impregnated (see Table I). Copulation was considered unsuccessful if a male seized and clung to a female but because of a faulty position or her struggles, failed to make connection.

females over a period of 10 days. After copulation the females were removed from the cage and their spermathecae were dissected and examined. If sperm were present the females were considered fecundated. The results are shown in Table I. From the Table it will be seen that over a 10-day period, one male copulating with 17 virgin females succeeded in impregnating 6 of them (one of the mated females was not dissected). It is apparent that a male will continue to copulate though no sperm are introduced into the female and the fact that a male copulated with a female does not imply that she has been impregnated. Also, we can conclude that after several initial copulations the male rids himself of sperm, but more spermatozoa are produced after a varying length of time. Bacot (1916) indicated that one male *aegypti* fertilized 10 out of 21 females and the maximum period during which the sperm remained alive and active in the spermathecae of the female was 62 days; the female in question laid further batches of eggs after this period, but none were fertile.

THE PRESENCE OF SPERM IN THE SPERMATHECAE

The spermathecae of *aegypti* females consist of 3 sclerotized, spherical, pigmented reservoirs lying ventrally within the eighth segment. The central spermatheca is larger than the 2 lateral ones which are of equal size. Each reservoir has its own colorless duct which unites into a common duct leading into the atrium (expanded distal extremity of the oviduct). During oviposition the spermatozoa travel from the spermathecae along the ducts and each egg is fertilized as it passes through the atrium (Marshall, 1938). The presence of sperm in the spermathecae and blood in the stomach apparently are the only prerequisites for the inception of ovulation in *aegypti* (McGregor, 1931). Macfie (1915) believed that fertilization must precede the blood meal before eggs will be laid. Gordon (1922) showed that a female which has not had a blood meal may lay eggs which do not hatch.

Ten females 11 days old, and which had been with males from the time of emergence, were dissected and their spermathecae examined. In all individuals only 2 of the 3 reservoirs contained sperm; these were the large central one and a smaller lateral spermatheca. With a strong light through the compound microscope, the 2 spermathecae filled with sperm were seen to be distinctly more opaque than the empty reservoir. However, all 3 spermathecae were crushed in order to make certain of the presence or absence of sperm.

Females will lay fertile eggs after one copulation and the following experiment was performed to determine if all 3 spermathecae ever became filled with sperm. Fourteen virgin females were mated once, each with a different virgin male, and their spermathecae were removed and examined for sperm ½ to 11 hours after copulation. The results were as follows: 2 females had no sperm in any of the reservoirs; 8 showed only the large central spermatheca and one lateral reservoir filled with sperm, the third being empty; of the other 4 females, 2 of each of their spermathecae were filled while the third small spermatheca may have had a few sperm present. In a second experiment, 9 females were placed individually in a cage containing many males and after copulating twice were removed and their spermathecae dissected and examined

three-quarters to 1½ hours after mating. Of the 9, 2 females may have had sperm in all 3 reservoirs but the number of sperm in 1 of the 2 small spermathecae was so small that it was difficult to decide whether sperm were originally present in this reservoir or whether they came from the other 2 crushed spermathecae. The other 7 females had only the central and 1 lateral spermatheca filled with sperm, the third being empty. With 1 or 2 copulations 1 of the lateral reservoirs generally does not become filled with sperm. The significance, if any, nor the reason for this phenomenon is not known.

THE ROLE OF SOUND PRODUCED BY THE WINGS OF THE FEMALE DURING FLIGHT

Males can recognize a female in flight for one flying close by a resting male often induced him to leave the side of the cage and pursue her. Apparently odor is not a factor in the mating behavior of *aegypti*. Although a resting female may be surrounded by males, some being so close that they touch her, never were any resting females seen to induce a male to attempt copulation. Also, males which readily mated with flying females were indifferent to the crushed bodies of these females. The most convincing evidence that odor is not a factor in locating the female was shown by the fact that male *aegypti* are not only attracted by certain sounds but also show a characteristic mating response to these sound frequencies (see p. 311).

Flight of the female is important in activating and attracting the male, and also in permitting him to assume a position during flight which will permit copulation. The importance of the sound produced by the female in flight was demonstrated in the following manner. A very fine wire was looped around the neck of a female or a piece of thread was shellacked or glued to her thorax and she was suspended in the middle of a cage full of males. The cage was then agitated to induce the males to fly. As long as the female hung from the wire or thread without vibrating her wings, the males remained indifferent and flew about her. Occasionally a male would land on the female but no attempt was made to copulate as long as the female's wings remained motionless. However, as soon as she began to vibrate her wings, the males flying nearby immediately seized her and attempted to copulate. Motion alone was of little importance since flying males remained indifferent to the suspended female (provided her wings were motionless) though the thread to which she was attached was swung to and fro among them. The wire or thread holding the female was also looped around a glass rod, instead of being suspended from the cage, and the female brought in close proximity to the resting males with similar results; the males did not attempt to copulate until she vibrated her wings. Lutz (1924, p. 337) noted no attraction of male mosquitoes to flying females confined in a widemouthed jar. He did not indicate the species observed and it is quite possible that Lutz was dealing with a form, like *C. pipiens* or *A. quadrimaculatus*, which mates periodically. The males of these mosquitoes become sexually active at certain periods of the day but at all other times they ignore the females.

Landois believed that mosquitoes produced sounds in two ways, one by

movement of the wings and the other by air being forced out through the thoracic spiracles (Howard et al. 1912, p. 114). The latter view has since been disproven. Shipley and Wilson (1902) described a stridulating organ on the basal part of the wings of *Anopheles maculipennis* which they considered to be the principal sound-producing organ. They quoted the work of Perez who showed experimentally that the stigmata take no part in the production of sound and that ". . . in both Diptera and Hymenoptera the buzzing is due to two causes, one, the vibrations due to the articulation of the wing which constitutes the true hum and the other the beating of the wings against the air, whose effect more or less modifies the first." Bellesme (quoted by Shipley and Wilson, 1902) confirmed the work of Perez and noted that of the two sounds emitted, one deep and one acute, the latter is usually the octave of the former. Shipley and Wilson believed that the stridulating organ at the base of the wings was responsible for the higher note. Pemberton (1911) also performed experiments which confirmed the fact that the buzzing of the wing bases, in Diptera and Hymenoptera, or a striking of them against an adjoining portion of the body wall produced the sound. Kahn, Celestin, and Offenhauser (1945) determined that sounds emanating from the mosquito were produced (1) during flight, (2) by beating the wings when at rest, and (3) by rubbing the hind tarsi against the wings. Pure "bird-like" sounds were recorded but their origin was not determined. Shipley and Wilson (1902) suggested the possibility that the halteres of the mosquito may function secondarily in producing a sound; the halteres may be responsible for the "bird-like" sounds recorded by Kahn et al.

It is apparent that the sounds produced by female *aegypti* while at rest are of no significance in mating since males ignore resting females and attempt to copulate only when the female is in flight or vibrates her wings. Banks (1908, p. 246) stated that specimens of *aegypti* confined in small jars ". . . have been seen to copulate while the female hangs from the gauze covering the vessel, the male always approaching her from the ventral surface." If this statement can be taken literally, the male initiated copulation while the female was at rest. Never in our observations was a male seen to initiate copulation with a resting female. Kahn et al. (1945) induced males to turn their antennae and hypopygia in the direction of a recorded female sound but they did not state what female "call" was used as the stimulus.

Female *aegypti* with about a third of the outer end of each wing amputated were capable of flight and males readily copulated with them. Female wings were cut off, bilaterally, at various distances from their bases and these mosquitoes were suspended in a cage containing males. Males were attracted to and copulated with females which had only small stumps of the wings vibrating. Males were attracted also to females vibrating both wings but lacking halteres. The halterectomized mosquitoes were suspended in the cage since they could not fly, and when they vibrated their wings males immediately seized them and copulated. Males ignored wingless females.

Nuttall and Shipley (1902) found that by cutting off more and more of

the wings of *A. maculipennis* the sound decreased in volume and the note rose progressively. When the wings were cut off quite closely a very high pitched note of slight intensity remained. The sounds produced by the cut wings of *aegypti* are different in pitch from those made by normal females during flight. The fact that males copulate readily with females possessing only a part of their wings indicates that (1) the males are capable of being stimulated by varying pitches produced by the female in flight, or (2) the males are being stimulated by the sound produced by a stridulating organ located at the base of the female's wings. Proof that the male responds to various pitches will be shown in the next section (see p. 282) and under the study of the behavior of males to mechanically produced sounds (see p. 326).

Few workers have attempted to determine the frequencies of the sounds produced by mosquitoes but it is generally known that the sound differs with the species and that the males in flight produce a sound which is distinctly higher in pitch than that made by the females of the same species. Nuttall and Shipley (1902) believed that this difference in the sounds of the sexes was due to the fact that the male wings were markedly narrower and shorter than those of the female. Weber (1906), Banks (1908, p. 248), Kahn et al. (1945), and others have been able to recognize different species of mosquitoes by the sounds they produced in flight. Kahn et al. successfully recorded the sounds given off by *A. aegypti, A. quadrimaculatus, A. albopictus,* and *C. pipiens;* these sounds were faintly audible or completely inaudible to the human ear and ranged from 250 to 1500 cycles per second. Nuttall and Shipley (1902), with the aid of tuning forks, determined the frequencies of the sounds produced by the males and females of *A. maculipennis* in flight. They found that in both sexes the pitch of sound was higher when the mosquitoes had fed, the greater the meal the higher the note. One unfed female gave an abnormally low note of about 175 vibrations; three others produced sounds from about 240 to 270 vibrations per second. Three unfed males gave notes corresponding to 880 vibrations though overtones were heard. Goeldi (1905) using a zither and tuning forks concluded that the sound made by female *aegypti* in flight was one of 480 vibrations while that of the male was 880 cycles. He indicated also that the extent of dilation of the abdomen with food influenced the pitch of the sound produced.

Chadwick (1939a) pointed out the fact that matching an insect's flight sound with the tone of an instrument of known frequency (i.e. tuning fork, etc.) is inaccurate since "The relation between the pitch of an insect's tone and the frequency of its wing motion is still not completely understood, and in many cases it appears likely that harmonics may be mistaken for the fundamental. For this reason, and because of difficulties thought to be introduced by the Doppler effect, not much attention has been given to this method by later workers." Chadwick introduced the use of the Edgerton stroboscope for the study of insect flight and employed this instrument in determining the frequency of wing-beat in *Drosophila* (1939b, 1943; see also Reed et al. 1942).

In the present work, the frequencies of sounds made by mosquitoes were not determined but sound recordings were made of flying individual males

and females of *A. aegypti* and *A. quadrimaculatus*. The pitch varied not only with the sex, species, and extent of feeding, but also with age. Recently emerged males and females produced sounds in flight which were decidedly lower in pitch than older individuals. The female with the unusually low note of about 175 cycles, mentioned by Nuttall and Shipley, had probably recently emerged. The recordings indicated that the pitch of sound produced by an individual mosquito during flight is variable.

The majority of observations concerning the mating of mosquitoes indicates that copulation generally is initiated in flight and it is probable that in these species sound plays an important rôle in mating behavior. However, there are several instances in the literature which indicate that sound is not or may not be significant in the copulation of certain species of mosquitoes. The New Zealand species *Opifex fuscus* Hutton, originally described as a tipulid (Hutton, 1902) was later shown to be a culicine mosquito by Edwards (1921). Edwards (1921) and Miller (1922) described certain morphological peculiarities of the male, mainly connected with secondary sex characters, and of possible biological significance. The male antennae are sparsely feathered and very similar to the female's; his front pair of legs are short, stout, and with very long, simple tarsal claws. The remarkable mating behavior of this species departs markedly from that known to occur in other mosquitoes. Kirk (1923) was the first to describe this behavior and his observations were summarized later by Edwards (1926). Briefly, the mating behavior is as follows: The males of *Opifex* rest on the surface of pools and within 10 to 20 minutes after emergence, seek out, and by means of their anal forceps, seize a mature pupa. The male ruptures the pupal skin and works his anal forceps backwards, extending the slit. The imago gradually emerges with two or three abdominal segments of the male within the pupal skin. If the emerging imago is a female, connection is made before her abdomen is quite clear of the skin, and when emergence is complete copulation is concluded in the end-to-end position, the mosquitoes resting on the water. The act lasts 3 to 7 minutes and was observed to last over half an hour. The proboscis and front claws of the male are used to ward off rival males after a pupa is seized. Females which emerge unaided, as a rule are unmolested by males. It is apparent that the sound produced by the female of *Opifex* during flight plays no rôle in mating behavior.

A. G. Richards (1941, p. 213) briefly described the copulation of a stenogamic autogenous strain of *Culex pipiens* and noted that the male rested on the side of the aquaria ". . . and the female seemed to be the aggressor since she flew around the male and eventually came to rest on top of him. Copulation ensued, the male appearing passive throughout the entire performance." Tate and Vincent (1936, p. 136) found the opposite to be the case for they noted that males of the English autogenous race of *C. pipiens* settled on and forced their way beneath resting females, copulation taking place with the females at rest. In either case, if copulation was initiated with one of the sexes at rest then the sound produced during flight was not the mating stimulus. Jobling (1937) reared several species of mosquitoes, including the autogenous race of *pipiens*, in complete darkness. This species was able to produce a

second generation in darkness and Jobling (pp. 473-474) stated, "The male cannot see the female in complete darkness; therefore it is attracted to it either by the scent, or the sound which the female may produce, or by both of these factors."

Cambournac and Hill's (1940) observations on the mating of *Anopheles maculipennis atroparvus* raise a similar question. When the males of this species become active they fly along the wall, "attack" resting females, and mate with some of them. "The preliminary attack, when the male momentarily touches the back of the female, apparently determines whether the latter is receptive. If so, the male moves around and up under the female, the latter accommodating herself to the efforts of the former by flying or half-flying with the forelegs still against the wall." It is difficult to imagine how the male recognizes the female by momentarily touching her back. However, if his touching her induces her to fly or "half-fly," the momentary vibration of her wings probably would be sufficient to induce the male to copulate. This also may be the case in Tate and Vincent's observations on the autogenous race of *pipiens*.

It is possible that the structure of the male antennae offers some indication of the mating behavior of different species. Edwards (1941, p. 35) though actually not observing the mating behavior of *Harpagomyia* did record the fact that males may hover around a resting female, ". . . the non-plumose antennae of the male suggest that mating swarms are not formed in the air as in most other mosquitoes." Among our mosquito fauna, at least two species have their male and female antennae more or less similar in structure in that the male antennae are sparsely plumose. These are *Deinocerites cancer* Theob. and *Uranotaenia lowii* Theob. The male antennae of *Deinocerites* are not only sparsely feathered but the presence of sensilla hairs on all the flagellar segments make them still more similar to the female's. Little is known about the mating behavior of *D. cancer*. Though observations on the biology of this species have been recorded by Knab (1906b), Duffy (Carpenter et al., 1946, p. 277), and Grabham and Low (Theobald, 1903, p. 279), the attitudes assumed and whether or not copulation was initiated in flight was not recorded. The observations made by Fisk (1941) on the behavior of *Deinocerites spanius* (D. and K.) also did not indicate whether mating was initiated during flight nor was the mating position described. Pratt (1946) collected copulating pairs of *U. lowii* resting on *Lemna* fronds or floating vegetation on the surface of their breeding places. It was not indicated whether copulation was initiated during flight or at rest.

The majority of observations concerning mosquito mating behavior have been made on *Anopheles*, *Aedes*, and *Culex*. Paine (1934) noted one instance of coition of *Megarhinus splendens* which lasted two minutes and took place with the pair settled. Hovanitz (1946) described the mating behavior of several species of South American *Haemogogus* mosquitoes. Practically nothing is known of the mating behavior of other genera.

ATTEMPTED COPULATIONS BETWEEN AEGYPTI MALES, AND BETWEEN AEGYPTI MALES AND OTHER SPECIES OF CULICIDAE

Occasionally male *aegypti* were seen to attempt copulation with other males. However, this was not a common occurrence when both sexes of about the same age were in a cage. When large numbers of males were isolated from females, the frequency with which males seized others of the same sex was increased greatly. One cage containing several hundred virgin males was disturbed for half an hour and during this period 59 pairs of males were counted as they dropped to the floor of the cage and attempted to copulate (those that did not drop to the floor were not counted). In most instances the aggressive male would flex his abdomen in a definite effort to make connection with his male partner. When the males were not induced to fly by agitating the cage the number of attempted copulations was less than the above figure, this chiefly being due to the fact that the mosquitoes tend to rest on the sides of the cage. The same males in which the above 59 attempted matings were noticed, were observed for one-half hour with only an initial jarring of the cage. Only 11 attempted copulations took place and these occurred during the first 10 minutes after the cage had been moved on the table. Comparatively few mosquitoes were in flight which indicated that these attempted matings were not due to accidental collisions in mid-air which might be possible when many mosquitoes were flying.

While observing a cage containing virgin males only, mostly 5 days old, an unusually large number of attempted copulations was seen to take place. Two days before, a beaker containing several hundred male pupae had been placed in the cage and the adults had begun to emerge. Upon removing the struggling pairs, it was found that in almost all cases one was a young male only recently emerged (shown by the fact that the genitalia were rotated only slightly or not at all). The following experiments were performed. Eighteen non-virgin males, 3 days old, were placed in a cage and disturbed for 15 minutes. During this period only one attempt at copulation was observed. Four young males the genitalia of which had not yet begun to rotate were introduced into this cage and the mosquitoes were disturbed. Immediately the young males were seized by the old ones which attempted to copulate. The cage was disturbed for 15 minutes during which time 98 attempted copulations were made by the old males upon the young ones; the young males could be recognized by their sluggish walk and flight. In the second experiment, 12 virgin males, 4 days old, 2 virgin females, 4 days old, and 2 males, 2 hours old, were placed in a cage together and the mosquitoes were disturbed for 15 minutes. There were 55 attempted copulations between the 4-day-old males and females (these were not divided into successful and unsuccessful copulations) and 49 attempted copulations between the 4-day and 2-hour-old males. Thus it is obvious that old males cannot differentiate between very young males and old females, both being about equally "attractive." Usually an old male would attempt to copulate with a young male whereas several males would pursue a female.

The sound of the male mosquito in flight is much more high-pitched than

the female. The pitch of the sound of a male is largely dependent upon the number of vibrations per second of its wings. The flight of recently emerged males is very slow and the pitch of sound produced by these males is distinctly lower than that made by older males. The difference between the pitch of sound made by old and recently emerged males is clearly heard in a very quiet room. Since the sound of the female is the external stimulus which induces male mating behavior, the inability of sexually active males to differentiate between young males and old females is due to the fact that the sound of the young male is reduced to a frequency which resembles the female, or falls within the range of sound which acts as a mating stimulus.

Since *aegypti* males respond to a range of sound rather than to a fixed pitch, and since sound appears to be the only external stimulus required to induce mating, then theoretically males of *aegypti* should attempt to copulate with other species of mosquitoes if the sound made by them falls within a stimulating range. The behavior of *aegypti* males towards several species of Culicidae collected in the field, was observed in the laboratory. Various species of mosquitoes were introduced into an 11″ x 11″ x 15″ cage and their relative "attractiveness" was determined by observing whether or not male *aegypti* would attempt to copulate with them, after the mosquitoes were disturbed and induced to fly. The findings are summarized in the following table:

Species	Behavior of male *aegypti*
1. *Anopheles quadrimaculatus* Say	Males and females pursued by virgin and non-virgin *aegypti*. Attempts to seize the male anopheline which produces a sound of higher pitch than the female appeared to be less persistent than attempts to seize the female. The *aegypti* male had difficulty in seizing the female *quadrimaculatus* which has very long legs, but if he did manage to cling to her a definite effort was made to clasp her genitalia. This was demonstrated by suspending a tethered female the legs of which had been amputated at the apices of the femora so that she could not struggle with the males when they seized her.
2. *Anopheles punctipennis* (Say)	Males and females very attractive to 2½ day-old virgins.
3. *Culex restuans* Theob.	Females appeared to be little or not at all attractive.
4. *Culex pipiens* L.	Males very attractive to 4½-day-old virgins. Engorged female *pipiens* were attractive to 2½-day-old virgin males; non-engorged females appeared less attractive than male *pipiens* to 7-day-old virgin *aegypti* males (feeding raises the pitch of sound produced by flying females).
5. *Aedes trivittatus* (Coq.)	Females very attractive to 4½-day-old virgins.

6. *Aedes vexans* (Meig.)	Males and females very attractive to 7-day-old virgins.
7. *Psorophora horrida* (D. and K.)	Females very attractive to 4½-day-old virgins.
8. *Psorophora ciliata* (Fab.)	Virgin *aegypti* males, 2½ days old were indifferent to a female of this culicine.
9. *Psorophora confinnis* (Lynch-Arr.)	Males very attractive to 6-day-old virgins; females appeared less attractive than males to 7-day-old virgin male *aegypti*.

An examination of the foregoing table shows that male *aegypti* will attempt to copulate with any Culicidae regardless of sex, the sound of which made during flight falls within the range which acts as a mating stimulus. The sound produced by the large female of *P. ciliata* apparently falls outside of this stimulating range so that she is unmolested by male *aegypti*.

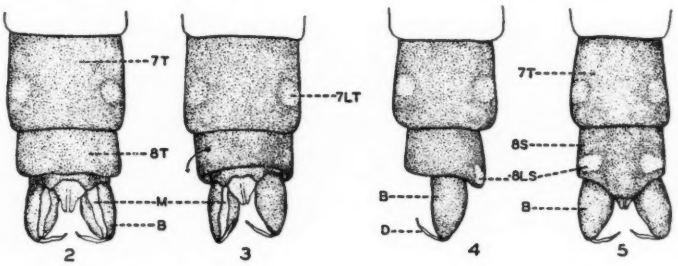
Richards (1927, p. 316) observed a male syrphid, *Merodon equestris* F., attempt to mate with a worker honey-bee; *Eristalis* males behaved in a similar manner, pursuing ". . . any insect which has a bee-like hum, such as they make themselves, . . ." Though Gruhl (1924) believed these acts due to errors of sight, Richards suggested that sound was equally important in this behavior. The present experiments showing that sound is the mating stimulus for *aegypti* males and that sexually active males cannot differentiate young males (the pitch of which produced in flight is lower than that of older males) from old females, but can distinguish between old males and females, indicate that sound is the chief, if not the only factor responsible for this behavior. All of these observations emphasize the fact that odor plays no part in the sexual behavior of *aegypti* and also that the male does not respond to a single fixed pitch but is capable of being stimulated by a range of sound frequencies.

ROTATION OF THE MALE GENITALIA AND AGE OF MALE AT FIRST COPULATION

At emergence, males tend to rest on the surface of the water for a few minutes and shortly afterwards fly to the sides of the cage where they remain quiescent for some time. Male mosquitoes are said to have a "hypopygium inversum" (Feuerborn, 1922) or "terminalia inverted" (Hardy, 1944). Christophers (1915) mentioned inversion in mosquitoes and showed that in male Culicidae the genitalia undergo a rotation of 180 degrees so that eventually the segments posterior to the seventh occupy a position diametrically opposite that which they first occupied at emergence. The rotation in the species of mosquitoes studied by Christophers took place within 24-48 hours after emergence. Marshall and Staley (1932) found that hypopygial rotation to 180 degrees reached completion when the air in the mosquito mid-gut had been expelled into the oesophageal diverticula. Marshall (1938, p. 289) stated that "In the case of at least many species, laboratory observations have shown that the males are able to mate as soon as the rotation of the hypo-

pygium is completed—a process which takes from twelve to twenty-four hours."

Ninety-two male *aegypti* were isolated at emergence and examined at intervals to determine the length of time required for rotation of the genitalia. At various intervals some of these males were introduced into a cage containing females to determine at what time after emergence copulation would occur. It was found that (1) rotation could be clockwise or counter clockwise, and (2) the rate of rotation was quite variable between different males (at room temperature). Rotation (Figs. 2-5) was first noticeable 1 to 4 hours after emergence and in some individuals the genitalia had rotated about 90 degrees as early as 5 hours after emergence. However, the rate was not uniform and rotation often ceased for hours after the genitalia had reached a certain angle. Most of the males copulated between 20 and 24 hours after emergence and in these individuals rotation was completed at this time. However, some copulated between 15 and 19 hours after emergence and in these the rotation also was practically complete. Apparently complete rotation was not necessary before males would attempt to copulate. Many males with a slightly less than 180 degree rotation were able to copulate successfully, assuming copulation was successful when connection with the female genitalia was made; several males the genitalia of which had rotated about



Figs. 2-5. Drawings of the terminal abdominal segments of male *Aedes aegypti* showing different stages in rotation of the genitalia.—2. Shortly after emergence; no rotation; note the membranous areas (M) of the basistyles (B) and the absence of the lateral pale scales on the eighth tergite; 3. Genitalia rotated about 45 degrees; the membranous area of the right basistyle is now invisible from above; the arrow indicates the direction of rotation; 4. Genitalia rotated 90 degrees. The lateral side of the basistyle is now seen from above, as is one of the lateral pale spots (8LS) of the eighth abdominal sternite; 5. Genitalia completely rotated 180 degrees; the original ventral surfaces of the basistyles are now dorsal and the membranous areas are no longer visible from the dorsal surface; the eighth sternite (8S) lies in the tergite position and its two lateral pale spots are more or less in line with those on the preceding segments. (Drawings outlined from photographs; the abdominal segments shown in Figs. 2 and 3 are slightly swollen and remain so for a short while after emergence.)

Abbreviations: B—basistyle; D—dististyle; M—membranous region of the basistyle; 7T—seventh abdominal tergite; 8T—eighth abdominal tergite; 7LT—lateral pale spot on the seventh abdominal tergite; 8LS—lateral pale spot on the eighth abdominal sternite; 8S—eighth sternite.

135 degrees attempted copulation and though most of them were unsuccessful one male did clasp the female genitalia and remained joined for several seconds. Only a few individuals required more than 25 hours for rotation to be completed. It will be recalled that *Opifex fuscus* males were observed copulating 10 to 20 minutes after emergence (Kirk, 1923) and this raises the question as to whether or not their genitalia had rotated within this short period.

Although the male genitalia must undergo a nearly 180 degree rotation before copulation can be successfully completed, it was evident that males would attempt to mate before their genitalia had rotated sufficiently to insure successful copulation. Induction of mating on the part of the male apparently is dependent upon the condition of the organs which receive the mating stimulus (i.e., the sound of the female in flight) and these structures are the antennae (see pp. 293 and 321).

Obviously the genitalia of both sexes are necessary for successful copulation and, in the male, must undergo rotation; however, the removal of the genitalia does not affect the mating behavior pattern. Non-virgin males, 3 days old, which had their abdominal segments posterior to the seventh removed, readily attempted to copulate with either normal females or females which had their genital segments removed.

AGE OF FEMALE AT FIRST COPULATION; "ATTRACTIVE" AND "UNATTRACTIVE" FEMALES

After emerging from the pupal skin, females also rest on the surface of water for a short while. Soon afterwards they fly to the sides of the cage and remain quiescent for several hours. Nineteen females were isolated just at emergence and placed in small 1″ x 3″ screen wire cages. Female genitalia do not undergo rotation. At various intervals these females were liberated for a 10-minute period into an 11″ x 11″ x 15″ cage containing 15, 4-day-old males. The mosquitoes were made to fly by jarring the cage at intervals. The earliest copulations occurred 50 minutes after emergence (4 females). Most of the matings took place about 105 to 145 minutes after the females had emerged. Males ignored 2, 3-hour-old females. It was apparent that very young females were not as attractive as older ones. Males ignored a very young female flying among them while they would mate instantly with one nearly their own age or older. Two examples can be noted: males attempted copulation 6 times (4 successful, 2 unsuccessful) with a 2-hour old female during a 10-minute period, while the same males attempted copulation 24 times (17 successful, 7 unsuccessful) with an older female during a similar exposure. Another female placed with males 135 minutes after her emergence copulated only once in 10 minutes while the same males copulated 17 times successfully and attempted 10 more unsuccessful matings during the same period with an older female. In both of the above cases the mosquitoes were made to fly by jarring the cage.

The stimulus which induces the male to copulate is the sound produced by the female during flight. With this in mind the relative unattractiveness

of these young females is explainable. The flight of the recently emerged female, like that of the male, is very slow and of short duration. The sound produced by the very young female in flight is lower in pitch than that produced by older females; the difference can be easily heard in a very quiet room and apparently the pitch of this sound is below the range which will stimulate the male to copulate.

Hinds (Howard et al. 1912, p. 275) observed that partly or fully fed *aegypti* females seemed more attractive to males than unfed individuals. Several experiments were performed using virgin females of the same age, some of which were starved since emergence, others fed on honey solution, and still others fed on blood. These females were about 2 days old when introduced and made to fly about in a cage containing males. It was found that starved females were no less attractive, during flight, than females the abdomens of which were distended with honey solution; that individuals with abdomens still distended with blood, the meal having been taken about 12 hours prior to the females being exposed to the males, also were no more attractive than starved females. However, when mosquitoes immediately after having had a blood meal were placed with males, copulations were comparatively few, because these recently-fed females were reluctant to fly although the cage was violently agitated. Even when made to fly the flight was of very short duration, the females moving slowly and heavily from one resting place to the next. Finlay (Howard et al. 1912, p. 260) observed that after a blood meal, *aegypti* ". . . is very sluggish and flies with difficulty; it seeks a hiding place where it remains quiescent during the process of digestion." Thus it is most unlikely that, in nature and the laboratory, females which recently had a blood meal will copulate. Wesenberg-Lund (1921) noted that males of *Aedes* (*Ochlerotatus*) *fletcheri* copulated with females the abdomens of which were distended with blood which, because of its black color, apparently had been taken some days earlier.

It is probable that Hinds was observing a difference in attraction due to a difference in age of the females. The partially or fully fed females in Hinds' observations may have been older than the unfed individuals which may have emerged recently. The sounds of the female vary with the extent of feeding but apparently still fall within the range which will act as a mating stimulus.

THE "MATING-STATE" IN MALES

Twenty-five newly emerged males were placed in a cage and after they were about 25 hours old, 10 females were introduced. Copulation took place immediately. The cage was observed and agitated several times every day (day and night) to determine how long the males would continue to copulate. Over a 10-day period (until the last male died) copulations occurred whenever the mosquitoes were made to fly. Thus once males were capable of mating and began to do so, they remained in a constant "mating state" throughout their life-time and copulated readily at any time, day or night, when the proper stimulus was supplied. Males show a definite mating response to mechanically produced sound long before they attempt to or are capable of

copulating; apparently young males may be in a mating-state though they do not attempt to copulate with females (see p. 324).

INCREASING THE FREQUENCY OF COPULATION

Howard et al. (1912, p. 275) quoted the works of French investigators who claimed that temperature influenced the sexual activity of *aegypti*. "When the temperature rises above 25° C. (77° F.) the males become very active and the females but rarely escape fertilization. When the temperature ranges between 20° and 25° C. (68°-77° F.) fertilization is still the rule and the proportion of unfertilized females is very small, but below this the proportion of unfertilized females increases rapidly with the reduction in temperature."

We have seen in the present study that once *aegypti* males reach a state wherein they are capable of copulating, usually about 15 to 24 hours after emergence, they continue to mate whenever females confined with them are made to fly in the cage. The stimulus which induces the males to copulate is the sound of the female during flight. Therefore any factor which induces both males and females to fly will automatically increase the frequency of copulation. Rudolfs (1922) made the following observations with regard to the behavior of *Aedes sollicitans* and *Aedes cantator* at various temperatures. At 17° C. they were inactive and at 20° C. they crawled about when the tubes containing them were shaken; at 25° and 30° they were lively and active; at 35° they flew about with very short rest periods; at 37° and 38° they flew constantly and at 39° they flew with short rest periods; between 40° and 46° the mosquitoes became less active, quiescent, and finally died. *Aedes aegypti* has been reported to show the greatest activity at about 28° C. (Howard et al. 1912, p. 271), temperatures beyond 39° C. being fatal while below 15° and 16° C. females became sluggish. Gutzevich (1931) stated that 28° C. was the optimum temperature for *aegypti* while *Aragão* (1939) believed that 27° to 32° C. was the optimum. High temperature does not increase sexual activity itself but by its thermo-kinetic[5] action induces the mosquitoes to fly, the sound of the female incidentally activating the males, which attempt to copulate.

Merely agitating a cage containing *aegypti* also increases "sexual activity" because both sexes are made to fly. Tate and Vincent (1936) found the same to be true of *Culex* for they noted that the slightest disturbance which causes the adults of autogenous *C. pipiens* to fly induces pairing. "For instance, if a cage of recently emerged adults is brought into the light from darkness, pairing begins actively and females may be surrounded by five or six males trying to pair. The same result arises from tapping the cage or blowing into it." This behavior is essentially the same as that of *aegypti*. Hovanitz (1946) in his study on *Haemogogus* mosquitoes noted it was difficult to distinguish between mating activity and general flight activity and that "In the cages it

5 Fraenkel and Gunn (1940) define kinesis as undirected locomotory reactions in which the speed of movement depends upon the intensity of the stimulation. The prefix indicates the physical nature of the stimulus.

was the species with the greatest general activity that showed the largest frequency of copulations and those with very little activity that could not be mated at all."

THE ROLE OF THE MALE ANTENNAE

Structure of the male antenna.—The male antenna (Figs. 6-8) consists of a scape (segment 1), pedicel (torus; segment 2), and 13 flagellar segments, making a total of 15 antennal segments. The scape is a narrow irregular sclerotized ring connected by a membrane to the greatly-enlarged pedicel (Fig. 6A). Child (1894) described the histology of the organ found in the pedicel of Nemocera (i.e. *Culex* and *Corethra*) and named the structure after Johnston (1855) who first suggested its sensory function.

Sections were made of the heads of newly emerged *aegypti*[6] males and it was found that Johnston's organ in this species is very similar to that of the males of *Culex pipiens* and *Corethra culiciformis* Degeer, described by Child (see Howard et al. 1912, pp. 29-33 and Plate 1). Figure 6 is a diagrammatic representation of a longitudinal section through Johnston's organ. Briefly, the structure consists of numerous sense cells (SC) with their distal ends attached to sclerotized processes (SP) of a circular plate (P) which arises from the base of the third antennal segment; the sense cells are arranged around the antennal nerve (AN) and are innervated by sensory fibers from this nerve. The distinguishing feature of chordotonal or sound receptive organs (sensilla scolopophora) is the presence of peg-shaped "sense-rods" or scolops at the apex of each sense cell and though Johnston's organ is generally classed with phonoreceptors, its sense rods are not typically scolopoid (Snodgrass, 1935, p. 528).

The first 11 flagellar segments are shortened though the first is somewhat longer than the following 10 segments (Fig. 6A). Each of the 11 flagellar segments has a subapical or nearly medial whorl of numerous long fibrillae or hairs which differ somewhat in length on each segment and become shorter towards the apex of the antennae. The fibrillae extend in all directions, dorsally, ventrally, and laterally; they form nearly a complete ring around the segment usually with a small gap between the hairs on one surface of the antenna (Fig. 7).

The first flagellar segment fits into the hollow of the pedicel and is darkly pigmented except for a small irregular colorless area below the subapical whorl. The pigmented area bears scales, minute spicules, and several long setae, which are shorter than the fibrillae forming the whorls. The apical portion of the segment directly above the whorl is pigmented and spicular. The second and third flagellar segments (antennal segments 4 and 5) are similar, each with an irregular pigmented basal spicular ring which is narrower than that found on the first flagellar segment; a colorless area below the whorls bears a few spicules or fine hairs and the portion above the whorls is spicular

6 Fixed in Carnoy's; cut at 5μ; stained with trioxyhaematin and Van Giesen's; some sections were also stained with Delafield's haematoxylin.

and pigmented. Flagellar segments 4 to 11 are more or less similar with the segments decreasing slightly in width towards the apex of the antenna. Each of these segments has a narrow, regular, basal pigmented ring, with the nearly colorless portion above the ring bearing a few small spicules. An apical pigmented spicular ring lies above each of the whorls. The region of insertion of the whorls on each segment is slightly enlarged. The twelfth and thirteenth flagellar segments (antennal segments 14 and 15, Fig. 8) differ markedly from the preceding ones. They are considerably elongated and the whorl of fibrillae of the twelfth is inserted on an expanded area above the base of the joint; a small clear area is found below the whorl as well as a very narrow pigmented ring at the base of the segment. The region beyond the whorl is darkly pigmented and possesses numerous long setae and many thin-walled sensilla arising from distinct depressions. The terminal segment is more loosely articulated than the others. Its whorl, which consists usually of 8 long fibrillae,

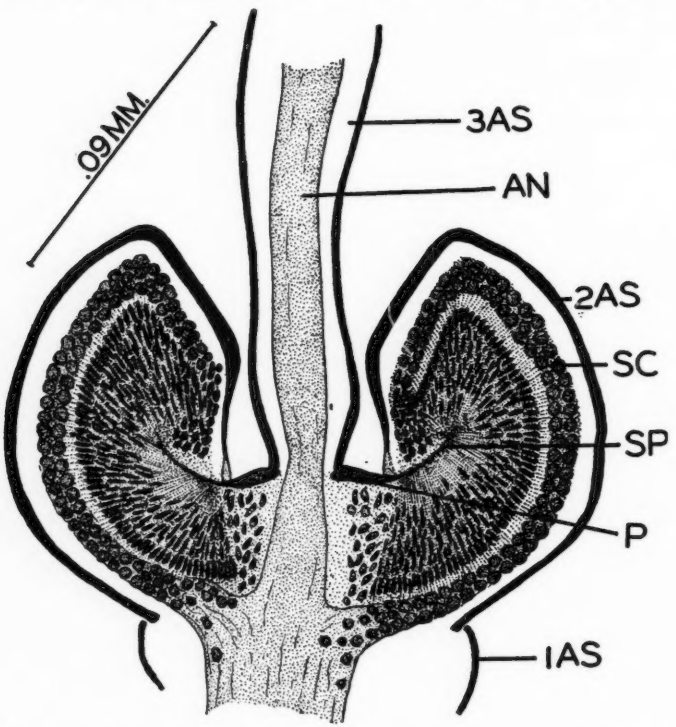
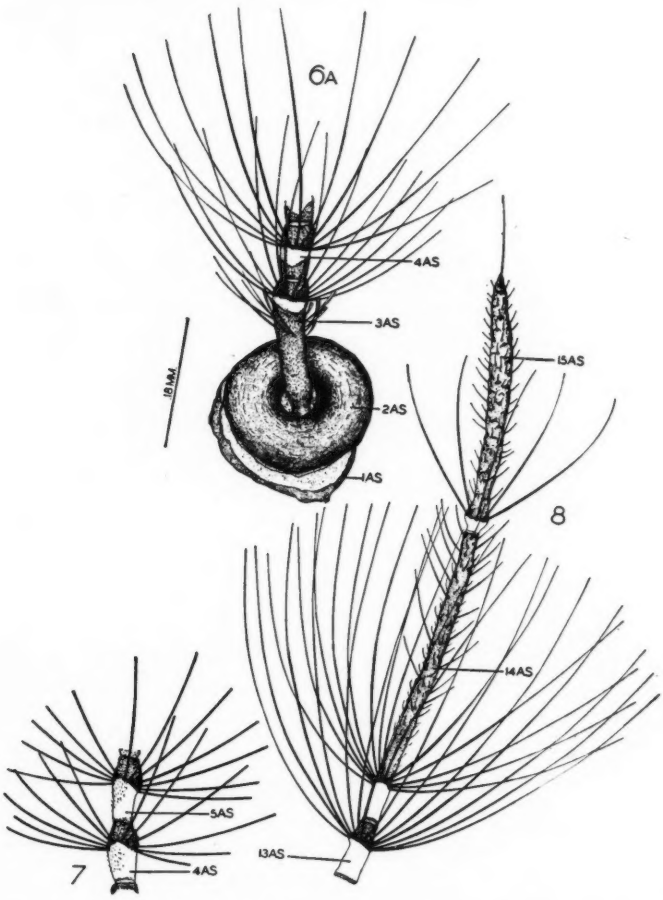
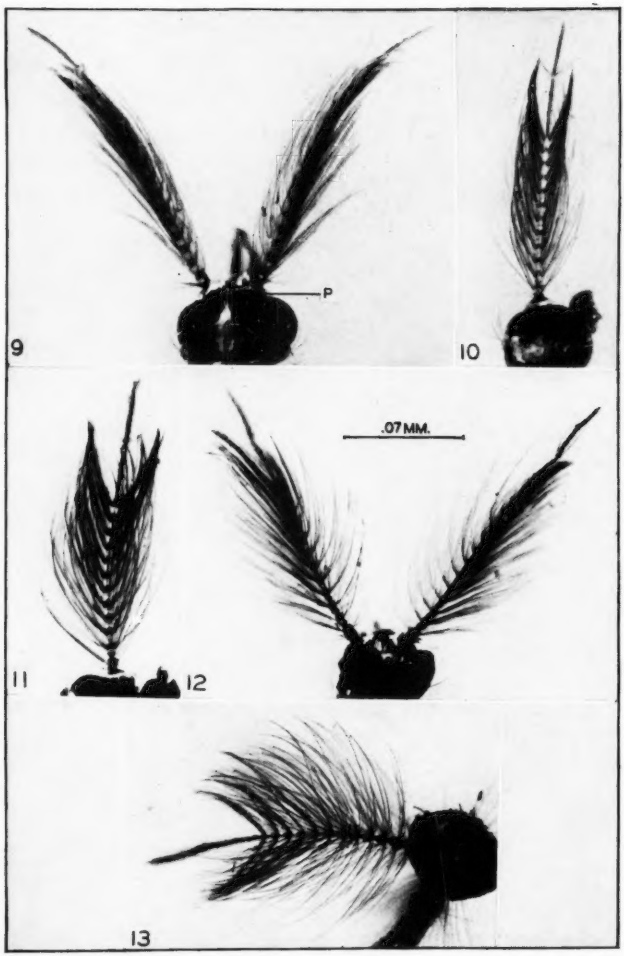


Fig. 6.—Parts of the antenna of male *Aedes aegypti*. Diagrammatic drawing of a longitudinal section through the first, second, and part of the third segments, showing the structure of Johnston's organ within the pedicel. (See Fig. 6A-8 for abbreviations.)



Figs. 6A-8. Parts of the antenna of male *Aedes aegypti*.—6A. First four antennal segments; 7. Fourth and fifth antennal segments showing the slight gap between the fibrillae; the full length of the hairs is not indicated; 8. Terminal three antennal segments. (All figures drawn to same scale.)

Abbreviations: AN—antennal nerve; P—plate; SC—sense cells; SP—sclerotized process of plate; 1AS—first antennal segment (scape); 2AS—second antennal segment (pedicel; torus); 3AS—third antennal segment (first flagellar segment); 4AS—fourth antennal segment; 5AS—fifth antennal segment; 13AS—thirteenth antennal segment; 14AS—fourteenth antennal segment; 15AS—fifteenth antennal segment.



Figs. 9-13. Heads of different aged males of *Aedes aegypti*.—9. Dorsal view of head shortly after emergence; most of the antennal fibrillae are recumbent; 10. Lateral view of head of a male of about the same age as the one shown in Fig. 9; note the wet-feather-like appearance of the antenna due to the recumbent hairs; 11. Lateral view of head, older than the one in Fig. 10, showing the fibrillae beginning to extend away from the shaft; 12. Dorsal view of head about 24 hours after emergence. Most of the fibrillae on the basal half of the antennae are well separated from the shafts; some of the fibrillae on the apical portions still cling together and are more or less recumbent; 13. Lateral view of head about 2 days after emergence. All of the antennal

is inserted near the base of the segment. The segment is completely pigmented except for the small area below the whorl, and is covered with many small depressions and their attached thin-walled sensilla; except for the basal whorl of fibrillae and a single seta at the apex, other long setae which are fairly numerous on the penultimate segment are absent from the terminal one. The lateral margins of the last two segments are distinctly irregular because of the depressions. The last two segments of the male antennae are somewhat similar to those of the flagellar segments of the female's in that their surfaces are covered with numerous thin-walled sensilla. The terminal two segments of the male antennae, bearing sensilla, possibly may be chemoreceptive organs.

In recently emerged males the fibrillar hairs forming the whorls tend to stick together and lie recumbent against the shafts, giving the antennae a wet-feather-like appearance (Figs. 9-10). As the males become older these fibrillae extend away from the shafts (Fig. 11). In 24-hour-old males most of the hairs on the basal half of the antennae are practically fully extended while those on the apical half may still be more or less recumbent and cling together (Fig. 12). In males 2 or more days old all of the antennal hairs usually are extended away from the shafts (Fig. 13) and generally they remain so throughout the lifetime of the insect; however, in some old males several of the whorls on the apical half of the antennae may be partly reclined. The fibrillae are curved and their tips point more or less towards the apex of the antennae.

The fact that male *aegypti* remain in a constant mating-state and copulate frequently and repeatedly at any time after they are capable of perceiving the sound of the female in flight, offers an excellent opportunity to study the behavior of individuals which have various structures, possibly used in mating, removed. After recovery from the operation the males are given an opportunity to copulate with females and their ability to do so is determined by counting the number of successful and unsuccessful copulations.[7] A ratio of 1 female to 5 males in a cage 11″ x 11″ x 15″ was found sufficient to determine the behavior of these operated males. In the following experiments on the antennae, and in the later ones on the legs, 15 males usually were used in each experiment and, unless otherwise stated, 3 females were introduced one or more times at different intervals after the males had been operated upon. The number of successful and unsuccessful copulations were counted, usually during a half-hour period, with the cage being agitated to induce flight whenever the mosquitoes came to rest.

The following series of experiments was performed to determine the behavior towards females, of male *aegypti* lacking parts or all of the antennal flagellar segments.

[7] See footnote 4, page 273.

fibrillae are extended away from the shaft (cf. Fig. 10). (Photographs of heads mounted on minuten pins; the mouthparts have been removed from the specimens shown in Figs. 9-12.)

Abbreviation: P—pedicel.

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Experiment 1.—To determine the behavior of males lacking the last two antennal segments.

As has been pointed out, the last two antennal segments differ markedly from the others. These segments were removed from both antennae of 15 virgin males. Some of the long hairs found subapically on the other segments were unavoidably severed or removed during the operation. Three females were introduced into the cage at 16 and 32 hours after the operation, for 2, half-hour trials. The results were as follows:

16-hour trial.—8 successful; 10 unsuccessful copulations.
32-hour trial.—42 successful; 34 unsuccessful copulations.

Conclusion. Males lacking the last 2 antennal segments still can locate the female in flight.

Experiment 2.—To determine the behavior of males after the bilateral removal of 9 flagellar segments.

In 24-hour-old males most of the fibrillae on the basal half of the antennae already are extended while many of those on the apical half still are more or less recumbent and tend to stick together (Fig. 12). However, these males locate females readily though one would imagine that recumbent and clinging hairs are not capable of being set into motion by the sound stimulus of the female (see p. 324). That these males can locate the female without the use of these hairs was shown in the following experiment.

Nine flagellar segments were removed from both antennae of 15, 3-day-old, non-virgin males. The remains of the antennae consisted of a scape, pedicel, and 4 flagellar segments and their attached fibrillae; since it was impossible not to cut or remove some of the fibrillae of the remaining segments, not all of the males had the same number of hairs remaining. Three females were introduced into the cage for 2, half-hour trials at 6 and 30 hours after the operation. At the 6-hour trial there were 5 successful and 2 unsuccessful copulations; at the 30-hour trial there were 11 successful and 17 unsuccessful matings.

Conclusions. Males lacking all but the scape, pedicel, and 4 flagellar segments of both antennae still are capable of locating and seizing the female in flight.

Experiment 3.—To determine the behavior of males after unilateral removal of 13 flagellar segments.

The flagellar segments, cut close to the pedicel, from one antenna were removed from each of 15 virgin males. The males were tested at 6, 21, 25, and 45 hours after the operation. The results of these 4, half-hour trials were as follows:

6-hour trial.—Only a few attempts were made to seize the female but none were successful.

21-hour trial.—3 unsuccessful attempts at copulation.

25-hour trial.—For the first 15 minutes, 3 females were used and there were 2 unsuc-

cessful attempts at copulation; twelve more females were introduced making a ratio of 1 male to 1 female and during the second 15 minutes there were 6 unsuccessful and 4 successful copulations.

45-hour trial.—9 unsuccessful and 11 successful copulations.

During the 6, 21, and 45-hour trials, 3 females were used.

Conclusions. It is apparent that the ability of the male to seize the female is markedly reduced by the removal of the flagellar segments of one antenna.

Experiment 4.—To determine the behavior of males after bilateral removal of all the flagellar segments.

The flagellar segments of both antennae were removed close to the pedicelli from 19 virgin males. After recovery these males were reluctant to fly and even when the cage was agitated they would cling to the sides. When flight did occur it was of short duration. Though difficult to disturb, the males walked about. Since males lacking the flagellar segments of one antenna made few attempts to copulate, 19 females were introduced into the cage so that the ratio of males to females was equal. Two trials were run 24 and 54 hours after the operation. No attempts to copulate were made, the males completely ignoring the females.

As a control, 15 males of the same age as those used in Experiments 3 and 4, during a half-hour period with 3 females made 67 attempts to copulate, 34 of which were successful.

Conclusions. The male antennae of *aegypti* are the organs used to locate the female in flight. The relative inactivity of flagellectomized males and the difficulty encountered in disturbing them indicate that the antennae perform a general stimulatory function. This behavior is comparable to that of *Rhodnius* (Wigglesworth and Gillett, 1934) which enter a state of "torpor" after amputation of their antennae. It was concluded that one of the functions of the antennae of *Rhodnius* was a kinetic one which maintained the nervous system in a responsive condition so that it would react readily to stimuli. This same kinetic function apparently is found in the male antennae of *aegypti*.

Some observations on the behavior of Anopheles quadrimaculatus.—Observations on *A. quadrimaculatus* were made with mosquitoes confined in a cage 11″ x 11″ x 15″, painted white on the inside. Copulations were infrequent and occurred only periodically. During the day both sexes of this anopheline were seen resting on the sides of the cage or the females flew about unmolested by the males. The male antennae are of particular interest for the whorls of hairs can be retracted (Fig.14) or extended away from the shafts (Figs. 15-16; cf. *aegypti* p. 291). During the day when the males are quiescent, their antennal fibrillae remain flattened against the shafts (Fig. 14). However, on several occasions, particularly at night, males with extended fibrillae (Figs. 15-16) were seen flying about the cage and attempting to mate with females. In one cage under observation, males were seen to begin to copulate at 6 p. m. and the following morning at 7 a.m., males with their fibrillae extended were still attempting to mate. Not all males with their fibrillae extended were attempting

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to copulate, but no males with these hairs flattened against the shafts were ever seen to pursue females.

Thus *quadrimaculatus* males reach a mating-stage cyclically and between these periods they ignore females and will not copulate. The extension of the male antennal hairs apparently is a good indication that they are ready to copulate and if a cage containing both sexes of this species is observed long enough, males with extended fibrillae will be seen attempting to copulate with females. *Quadrimaculatus* males which are in a mating-state will pursue female *aegypti* when the latter are introduced into the cage.

Concerning the antennae of *A. maculipennis*, Nuttall and Shipley (1901, p. 460) observed that "The captive males, during the daytime, usually carried the hairs closely applied to the shafts of the antennae, the hairs being extended (as in Plate I.) when they began to fly about in the evening. On one occasion three males extended their antennal hairs after being fed sugar and water, this being in the evening when they were active. One of them, on coming to rest, flattened down the hairs again with his fore-legs." These writers did not attempt to explain the possible significance with regard to mating, of the extension of the antennal hairs. Male *quadrimaculatus* with their antennal fibrillae recumbent, have been observed feeding on sugar solutions.

THE ROLE OF THE MALE LEGS

In addition to the antennae and genitalia, the male legs play an important part in mosquito copulation. Lamb (1922, p. 10) suggested that the male palps possibly have some rôle in mating, but this is unfounded since non-virgin males several days old, which had their palps and proboscides removed readily located females in flight and copulated. The palps and proboscis take no part in the mating behavior of *aegypti*.

The following experiments were performed to determine the mating behavior of males lacking various tarsal segments. The method of testing was similar to that used in the preceding antennal experiments.

Experiment 1.—To determine the behavior of males after unilateral tarsectomy of the front legs.

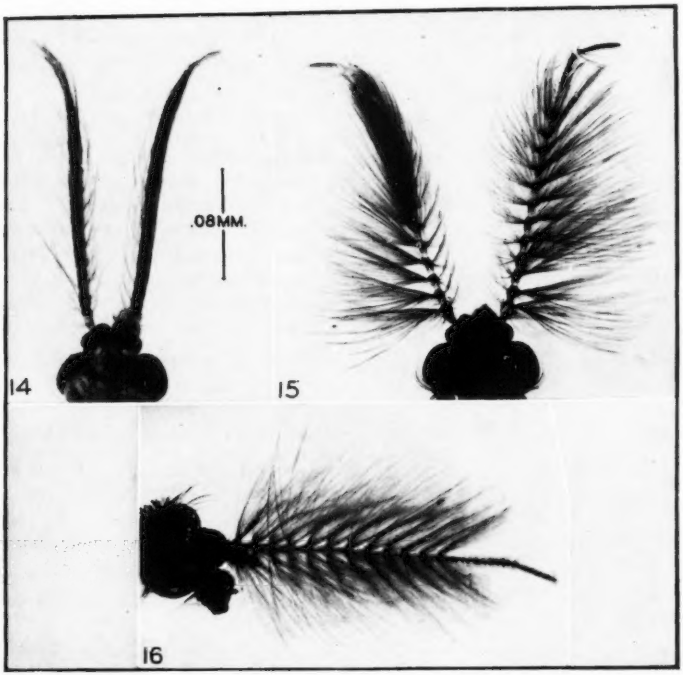
The tarsus was removed from one front leg from each of 15 virgin males. Fourteen hours later these males copulated 38 times successfully and attempted 37 unsuccessful matings with 3 females during a half-hour period.

Conclusion. Males can copulate successfully after the removal of one front tarsus.

Experiment 2.—To determine the behavior of males lacking both front tarsi, or the last segments of the front tarsi.

The males used in Experiment 1, already lacking one front tarsus, had their other front tarsus removed. Eighteen hours later the males copulated successfully only 3 times but made 100 unsuccessful attempts to mate.

The experiment was repeated using 15 other virgin males. Three females were introduced at 11 and 28 hours after the operation and there were 49 unsuccessful copulations during the first trial and 74 unsuccessful copulations during the second. No successful copulations occurred during both 30-minute trials. Almost invariably the male failed to gain a vertical pose. His middle legs managed to seize the female's hind legs but when she came to rest on the side of the cage the male was either on top, off to one side, or hanging beyond still suspended from her legs. Often while in the last position, with his back against the cage, he would flex the tip of his abdomen upward so that eventually he was on top of the female with his head facing posteriorly and his genital segments in a region above her wings. Sometimes when suspended low enough, the male attempted copulation from a tail-to-tail attitude, the heads



Figs. 14-16. Heads of males of *Anopheles quadrimaculatus*.—14. Dorsal view showing the antennal fibrillae recumbent against the shafts; note the thickened appearance of the shafts (cf. Fig. 15). Some of the hairs are extended; this occurred after the insect had been anesthetized; 15. Dorsal view showing the antennal fibrillae extended away from the shafts giving the antennae a plumose appearance. Some of the hairs on the apical half of the left antenna are somewhat recumbent; 16. Lateral view of the same head shown in Fig. 15. (Photographs of heads mounted on minuten pins; the mouthparts have been removed from all specimens.)

of both individuals pointing in opposite directions. However, the male never succeeded in making connection from an opposed attitude.

The last tarsal segments from both front legs of 15, 3-day-old males, were removed. During one trial 12 hours after the operation, 89 unsuccessful attempts at copulation were made, with 3 females. There were no successful copulations. The behavior of these males was similar to those lacking both entire front tarsi.

Conclusions. It is apparent that the last tarsal segments of the front legs are not necessary for the initial seizure of the female in flight, but these segments are vital for the male to assume the vertical pose necessary for successful copulation.

Experiment 3.—To determine the behavior of males lacking the middle tarsi.

The middle tarsi of 15, 3-day-old males were removed and 2 trials were made 6 and 27 hours after the operation, with the following results:

6-hour trial.—11 successful copulations; 66 unsuccessful copulations.
27-hour trial.—27 successful copulations; 39 unsuccessful copulations.

During the first trial, difficulty in mating apparently was due to the fact that the male, although able to seize the female, was "shaken" off just before or after the pair came to rest. Even males which managed to gain a ventral attitude, often were unsuccessful in copulating if the attitude was one which required manipulation of the female's abdomen. Several of the successful matings occurred on the floor of the cage where the male was in a position which merely required raising his abdomen to make connection with the female genitalia.

Conclusion. Males lacking the middle tarsi still are capable of copulating successfully.

Experiment 4.—To determine the behavior of males lacking the hind tarsi.

The hind tarsi of 15, 3-day-old males were removed and one trial was run 5 hours after the operation. Forty-five unsuccessful and 55 successful copulations occurred during the 30-minute period. These males easily seized the female in flight and readily assumed the vertical pose in spite of the absence of the hind tarsi.

Conclusion. The hind legs of the male take little part in copulation (see p. 270).

Experiment 5.—To determine the behavior of males after tarsectomy of the middle and hind legs.

The middle and hind tarsi were removed from 15 virgin males and their behavior towards females was observed 8 and 22 hours after the operation. The results were as follows:

8-hour trial.—14 successful copulations; 45 unsuccessful copulations.
22-hour trial.—10 successful copulations; 52 unsuccessful copulations.

Conclusions. The results are comparable to those obtained in Experiment 3 after removal of the middle tarsi. This is to be expected since it is evident from Experiment 4 that the hind legs play little part in copulation. The total of 24 successful copulations indicates, again, that the front legs alone are capable of seizing the female and are used in gaining the vertical pose.

Experiment 6.—To determine the behavior of males lacking the last segments of the front and middle tarsi.

The last segment of the front and middle tarsi of 15, 3-day-old males were removed and one trial was made 18 hours after the operation. In spite of repeated and numerous attempts, not one male was successful in seizing a female in flight.

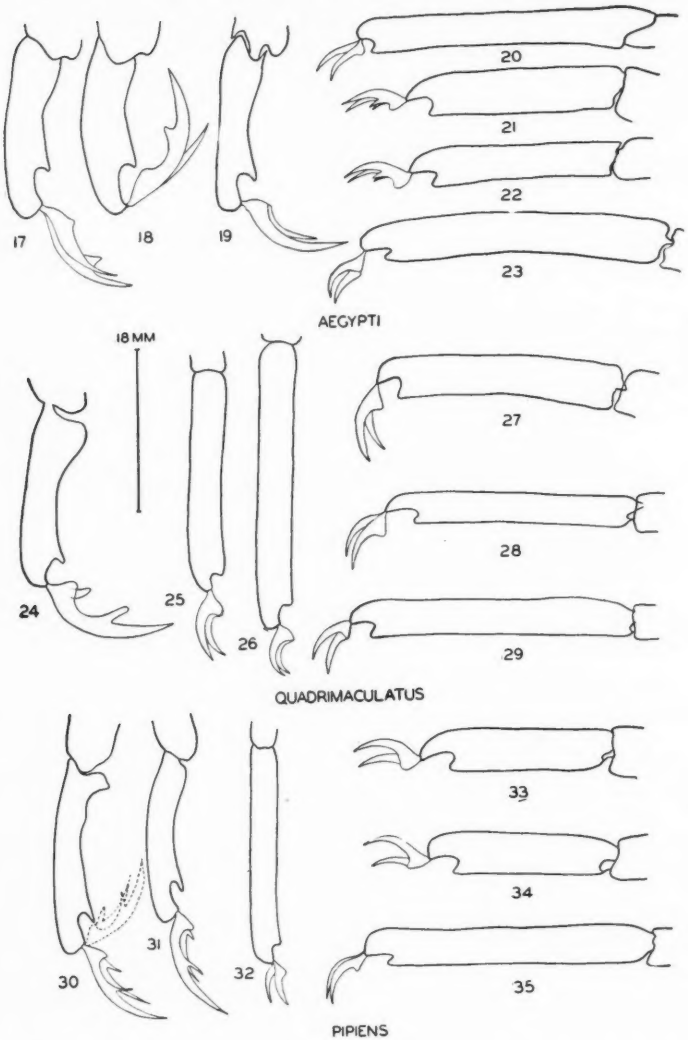
Conclusion. The last tarsal segments of the front and middle legs are absolutely necessary for the male to seize the female and to assume the vertical pose necessary for copulation.

GENERAL DISCUSSION OF THE ATTITUDES OF MOSQUITOES ASSUMED DURING COPULATION

The results of the experiments on the male tarsi may be summarized in the following table:

Type of operation	*Behavior towards female*
1) Removal of one front tarsus	Can copulate successfully.
2) Removal of both front tarsi	Male is capable of seizing the female but generally cannot copulate because of his inability to gain a vertical pose.
3) Removal of the last segment of both front tarsi	Similar to 2) above.
4) Removal of both middle tarsi	Can copulate successfully.
5) Removal of both hind tarsi	Can copulate successfully.
6) Removal of both middle and hind tarsi	Can copulate successfully.
7) Removal of the last segments of both front and middle tarsi	Male cannot seize the female and therefore is unable to copulate.

The ability of male *aegypti* to seize and hold the female is due to the greatly enlarged tarsal claws and the hollowed inner margin of the last tarsal segments of his front and middle legs (Figs. 17-19). Thus the part of the female femur, tibia, or tarsus which is seized by the male fits into this depression, and his claws are clasped over the margin of her appendage and hold it firmly in place. The hind tarsi (Fig. 20) are not at all suitable for seizing because of the small claws and the absence of the indentation along the inner margin of the last tarsal segment. For this reason de Riville's early observation



Figs. 17-35. Last tarsal segments and tarsal claws. 17-23: *Aedes aegypti*.—17. Male, prothoracic; 18. Male, prothoracic (tarsal claws retracted); 19. Male, mesothoracic; 20. Male, metathoracic; 21. Female, prothoracic; 22. Female, mesothoracic; 23. Female, metathoracic. 24-29: *Anopheles quadrimaculatus*.—24. Male, prothoracic; 25. Male, mesothoracic; 26. Male, metathoracic; 27. Female, prothoracic; 28. Female,

(see p. 268) that the male used the middle and hind legs for grasping the female is no doubt an error. He may have mistaken the pushing action of the male hind legs against the female abdomen or legs for one of grasping.

In mating, the primary orientation of the male depends upon his ability to perceive and determine the direction of the sound stimulus emanating from the female in flight. Once this has been accomplished, with the aid of his antennae, and he has successfully pursued and seized her, a second orientation becomes necessary. For if the male seizes the female in other than a venter-to-venter attitude, as generally happens, he must reorient himself before copulation can be successfully completed. This second orientation, which takes place while the male clings to the female, is largely accomplished with the aid of the front tarsi for when these are removed the male, in almost all cases, can no longer gain the vertical pose although still capable of clinging to the female with his middle legs. The behavior of these males lacking the front tarsi is also good indirect evidence that the normal male initially seizes the female in a dorsal position and then reorients himself while clinging to her. The middle legs appear to function largely in the initial seizure of the female, though the front legs are also used in this act. That successful copulations can occur in the absence of the middle and hind tarsi indicates that the front legs can perform, to some extent, both the function of seizing the female in flight and in gaining a vertical pose. The tarsal claws of the middle legs are large but not toothed (Fig. 19). The tarsal claws of the front legs are still more developed by reason of their greater length and toothed condition (Figs. 17-18). This structural difference may explain why males can copulate lacking their middle and hind tarsi, but not when the greater-developed front tarsi are removed.

Knab (1907b) compared the attitudes taken by the sexes of *Anopheles punctipennis* Say during copulation with that assumed by other species, chiefly *Culex pipiens*. He stated, "It seems remarkable that the attitude taken by *Anopheles* in copulation, end to end and facing in opposite directions, is identical with that of *Culex pipiens*. This remarkable agreement is significant when the structure of the tarsal claws is considered. In both forms it is practically the same and all the claws of the female are simple. In both cases the legs are not made use of in the copulatory act itself but only in the preliminary scuffle. Dr. H. G. Dyar has found that *Culiseta consobrinus* (= *Culiseta inornata* Will.), another form with simple claws and quite a distinct generic type, takes the same position in copulation. It is fair to assume that this is the mode of copulation in all the forms in which the female has simple claws. On the other hand it appears that in those forms in which the female has toothed claws the position in copulation is face to face, the pair clasping each other. The writer can confirm Goeldi's observation that *Stegomyia calopus* copulates in this manner. Dr. Dyar has observed the same method of copulation in

mesothoracic; 29. Female, metathoracic. 30-35: *Culex pipiens*.—30. Male, prothoracic (dotted line shows claws retracted); 31. Male, mesothoracic; 32. Male, metathoracic; 33. Female, prothoracic; 34. Female, mesothoracic; 35. Female, metathoracic. (All drawings outlined with the aid of camera lucida.)

Aëdes varipalpus Coq. One cannot avoid the inference that the difference in the structure of the claws is closely correlated with the mode of copulation. It would appear then that the structure of the claws is a purely adaptive one and therefore it cannot be considered of the deep classificatory significance attributed to it by Mr. Coquillet." *Aedes spencerii* Theob. and *Aedes fitchii* Felt and Young copulate face-to-face and of the former, Knab (1908) stated, they grasp ". . . each other with their long legs, the female in the upper position, the male back downward." Wesenberg-Lund (1921) noted that the male of *Aedes fletcheri* "dances" close to the ground, the female quitting the leaf on which she is resting, and when the sexes meet they grasp ". . . each other's fore and middle legs." In this species copulation also takes place in the facing position.

Earlier in this paper it was pointed out that the female legs were extended and usually held outstretched away from the body, during copulation; when a joined pair landed on the side of the cage the female used her front and middle legs to cling to the cloth while her hind legs were pushed upward by the action of the male's middle legs. Direct observation through a binocular microscope placed above the tethered females showed that the male seized the female, her legs taking no part in the action. Banks (1908, p. 246) also observed that the male seized the flying female ". . . with his fore and mid feet." He recorded no observations to the effect that the female grasped the male. Final proof that the female tarsal claws are not used in mating was obtained by removing the last tarsal segments from all the legs of 10 females. Males, introduced into the cage, easily seized these operated females, clinging to them in flight and copulating. The only difficulty occurred when the females tried to land on the sides of the cage. Removal of their tarsal claws prevented them from gaining a hold on the cloth side and they invariably fell to the floor where copulation usually was completed with the females astride the male (i.e. venter-to-venter). When the last tarsal segments from the front and middle legs only were removed from females, the results were more or less the same except that in some cases the females managed to cling awkwardly to the side of the cage with their hind tarsal claws and copulation was completed there.

It is understandable that an observer witnessing pairing in the field, with the naked eye, could be misled to believe that the female used her legs to cling to the male. Again Howard, Dyar and Knab (1912, p. 121) stated that "The attitude assumed during the copulatory act differs according to the structure of the claws of the female. In the forms with simple claws (*Culex, Anopheles*) the position is end to end, the pair facing in opposite directions. The forms in which the female claws are toothed copulate face to face, clasping each other with their claws." During coitus the female *aegypti* does not clasp the male to her. Perhaps Knab was correct in his assumption that all forms in which females have simple claws copulate end-to-end while those in which the claws are toothed copulate face-to-face. However, this difference in attitude, at least in *aegypti*, cannot be attributed to the fact that the female can clasp the male to her because of her toothed claws, for her legs, remain-

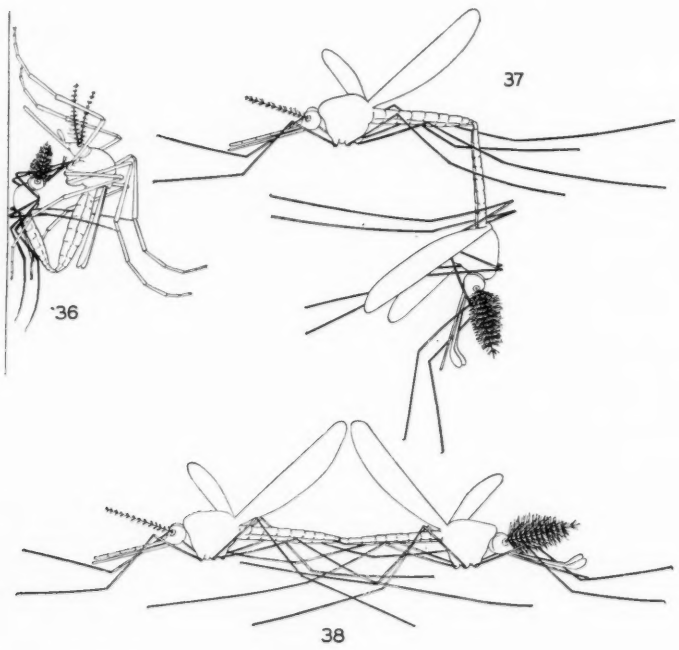
ing outstretched while flying about with the male, merely serve as structures to which the male can cling. It might be correct to state that face-to-face copulation is possibly characteristic of mosquitoes in which the tarsal claws of the female are toothed, and end-to-end copulation is characteristic of mosquitoes in which the females have simple claws, with no correlation with the function of these claws.

An examination of the female tarsi of *aegypti* (Figs. 21-23) reveals that the last tarsal segments of her front and middle legs (Figs. 21-22) are not at all suitable for clasping (cf. Figs. 17-19) in spite of the toothed character of the claws. These claws are small and the margins of the last segment are practically parallel; even when the toothed claws are retracted to their fullest extent they are actually no more useful for clasping the male than are the simple claws found in *Anopheles quadrimaculatus* (Figs. 27-28) or *Culex pipiens* (Figs. 33-34) females. That a toothed tarsal claw condition is not actually necessary for seizing is seen in the fact that male *aegypti* which have their front tarsi removed can still grasp the female (though they cannot gain a ventral position) with their middle tarsi, though the claws of the middle leg are long and simple.

Boyd, Cain, and Mulrennan (1935) photographed a pair of *quadrimaculatus* in the end-to-end position killed during copulation and stated "Copulation occurred in flight, the individuals assuming an end-to-end position and settling in pairs to the floor of the cage (Fig. 7)." In our 11″ x 11″ x 15″ cages, males would become active and begin to fly in the evenings, a behavior presumably comparable to the swarming of *quadrimaculatus* in the field (see Freeborn, 1923). Hackett and Bates (1936), Rao and Russell (1938) and others have observed swarming of *Anopheles* in insectary cages. In the cases of copulation seen in the laboratory, the male *quadrimaculatus* appeared to have great difficulty in seizing and clinging to the female long enough for him to clasp her genitalia. He seized her in flight, assumed the modified linear pose, but once connection was made with her genitalia the male released his leg hold and in so doing sometimes dropped to a pendant position for an instant (Fig. 37); he then passed to the opposed or linear position (Fig. 38) with the heads of the two facing in opposite directions. Sometimes the pair flew about with the male hanging head downwards. Copulation was of short duration and could be completed (1) in the air, (2) with the female clinging to the side of the cage, the male hanging head downward, or (3) on the floor of the cage with the mosquitoes resting on their legs, joined by their genitalia, and facing in opposite directions. After copulation was completed, the male released the female and flew away.

The mating positions of *atroparvus*, as reported by Cambournac and Hill (1940), were very similar to that of *quadrimaculatus* except that the former "attacked" resting females. Once the male *atroparvus* made connection with the female he released his hold after two or three seconds and ". . . the two remained 5 or 6 seconds in a straight line, tail-to-tail. If the pair is on the wall instead of in flight, the insects are quiet and the male hangs straight down with the wings outspread."

If one compares the attitudes assumed during copulation of *Aedes aegypti* (Fig. 36) and *Anopheles quadrimaculatus* (Fig. 38) the face-to-face position of the former and the end-to-end position of the latter can be correlated with the extent of development of the last tarsal segments and claws of the front and middle legs of the male. In *quadrimaculatus* the last tarsal segment of the middle leg (Fig. 25) is practically as poorly developed as that of the hind leg (Fig. 26), with the claws being comparatively small and the inner margin of the last segment lacking the depression found on the front leg. Only the male's front tarsi (Fig. 24) are suitable for seizing and holding the female. Thus one can easily reason that the reduced development of the middle tarsal claws of the male correspondingly reduced his ability to seize and cling to the female and this eventually resulted in the end-to-end position, an adaptation which after the initial scuffle with the female did not require the use of the male legs at all. We may also recall that because of their greatly developed



Figs. 36-38. Diagrammatic drawings showing the mating positions of mosquitoes.—36. *Aedes aegypti* in copula, the female at rest on the side of the cage, the pair in the face-to-face position (the legs of the male are blackened); 37. *Anopheles quadrimaculatus* in copula with the male in a temporary pendant position; 38. *Anopheles quadrimaculatus* in the final stages of copulation with the pair in the end-to-end position.

front tarsal claws, *aegypti* males are capable of copulating even after their middle tarsi have been removed. However, removal of the middle tarsi of *aegypti* did not result in that species assuming a tail-to-tail position during copulation.

There are certain observations in the literature which make it rather difficult to formulate a general hypothesis regarding the correlation of mating attitudes and the structure of the male claws. If we accept Knab's (1906a) observations that *Culex pipiens* assumes a tail-to-tail position during copulation we possibly have an objection to the hypothesis since in this species the front and middle tarsi are both about equally developed and suited for seizing (Figs. 30-31). However, the retracted front tarsal claw of male *aegypti* reaches almost to the base of the last tarsal segment (Fig. 18) while that of *Culex pipiens* does not (Fig. 30, dotted lines). This might make the *aegypti* legs more efficient than the *pipiens* legs in seizing and holding. Regarding the copulation of *C. pipiens,* Wesenberg-Lund (1921) stated "I never could directly observe that the mating position was end-to-end, as indicated by Knab (1912, p. 122); the insects were always curled up in a ball and very soon reached the grass. Here the act was accomplished in the course of a few seconds, after which the mosquitoes flew away."

A laboratory colony of *Culex pipiens* was established from larvae collected in the field. Reared adults were kept in a cage 24″ x 24″ x 30″ lined with white cheesecloth to facilitate observation. Feeding and mating was cyclical in this species and occurred early in the morning. At first, females would only feed on the arm between 3 a. m. and 8 a. m. Copulations were observed between 6 a. m. and 8 a. m. and a few attempted copulations took place at 3 a. m. The mosquitoes were more or less inactive at other times of the day; that is, the females did not take blood and the males would not copulate. After several generations, females would feed on blood more readily during the day and later in the evening, and males were seen to attempt copulation about 11 p. m. Unlike *quadrimaculatus* males, *pipiens* males were observed with their antennal fibrillae remaining extended during their sexually inactive periods; like *aegypti,* the males of *pipiens* were never seen with all their antennal fibrillae flattened against the shafts once these hairs had become extended.

Copulation of *pipiens,* in our laboratory colony, was always initiated on the wing, the male being the aggressor and seizing the female in flight. The male appeared to be less adept at seizing the female than was *aegypti;* many of *pipiens'* attempts were unsuccessful. The male seized the female, assumed a vertical pose, and then clasped her genitalia. They would fly about in the facing position for a brief period and then the male would release his leg hold and hang head downward while clasping the female genitalia, and thus be carried by her (similar to *quadrimaculatus* shown in Fig. 37). Copulation was completed in flight, the male releasing the female after a few seconds. In many instances pairs would fly about joined face-to-face and then separate; however, the action was so transient that it was difficult to determine if connection was made during the act. If a female came to rest with the male clinging to her in a face-to-face attitude, he would release her and fly away. If,

however, connection had been made and the male was in an opposed position suspended from the female's genitalia when she came to rest, the pair would remain attached by their genitalia for a brief period until copulation was completed. Apparently, before copulation could be successful, the male had to make connection while the pair was in flight and before the female came to rest. Bates (1941, p. 167) found anophelines behaved in a similar manner. Successful copulation took place in a comparatively small space since viable egg rafts were obtained from blood-fed *pipiens* kept with males in a cage 11″ x 11″ x 15″.

Marshall (1938) reared many generations of *Culex molestus* Forskål (*C. pipiens molestus* Forskål; Jobling, 1938) and found that pairing of the adults though invariably initiated in the face-to-face position was often concluded in the end-to-end one. Apparently Marshall refers to the fact that the pair may fly about joined, venter-to-venter for awhile before assuming the opposed position. This behavior is similar to our observations on *pipiens*.

Even *quadrimaculatus* may be said to initiate copulation in a face-to-face attitude since connection is first made with the pair flying in a vertical pose. However, the opposed position is assumed practically the instant connection is made; this differs somewhat from *pipiens* where the pair may fly about for awhile in the facing position before assuming the end-to-end one. Rao and Russell (1938, p. 400) described 2 cases of pairing of *Anopheles annularis* in which ". . . both members of the pair faced the same direction, towards the wind, one being above and the other below, legs interlocked, with tips of abdomen in contact." They noted a similar behavior on the part of *Anopheles stephensi* in a cage colony although this species also was observed to pair in the end-to-end position. The writers did not indicate whether or not they saw the individuals which copulated in the facing position separate without actually assuming an end-to-end one.

In discussing the positions assumed during copulation it is important that one sees the complete act and differentiates between the end-to-end position taken during actual copulation and an end-to-end position which sometimes may be taken briefly as the sexes try to separate after copulation is completed. The writer noted on several occasions that after mating, pairs of *aegypti*, in attempting to free themselves, momentarily assumed the linear position. Knab (1908) describing the copulation of *A. fitchii* states "Union takes place 'face to face,' the pair flying obliquely upward for several yards. Then the pair would either separate promptly or swing out end to end and struggle to disengage themselves. In this latter case both of them could be seen to jerk violently and rapidly in their efforts to free themselves, and the pair would slowly rise, but make no appreciable progress in either direction." An observer noting only the termination of the copulatory act might be led to believe that in species behaving in the above manner, the linear or end-to-end position is the one assumed during copulation; conversely, one seeing only the initiation of the act in those forms which copulate end-to-end would be led to believe that the facing position is characteristic for that particular species. *Aedes aegypti* normally copulate in the face-to-face position; *A. quadrimaculatus* remains only

momentarily in the facing position, the greater part of the act being performed in the opposed position; *Culex pipiens* apparently falls into a class between the above two species often spending part of the time in both positions.

A. G. Richards (1941) described the copulation of a stenogamic autogenous strain of *Culex pipiens* and noted that the females came to rest on top of quiescent males, copulation taking place with the male appearing passive throughout the act. If taken literally, Richards' description gives the impression that copulation occurred with the sexes in the typical female vertical pose, that is, the female astride the male with his dorsal surface against her venter. This seems highly unlikely considering the rotation of the male genitalia. Copulation in the female vertical pose occurs in insects in which the male hypopygium is normal (i.e. no rotation; see O. W. Richards, 1927, p. 336). In a personal communication (1946) A. G. Richards emphasized the fact that his observations could not be compared with Knab's since one was a field study while the other was made in the laboratory. Richards in his letter stated, "Mating in these cases was probably to a certain extent abnormal in the sense that relatively small containers were used and accordingly the data may not reflect the behavior to be found in nature where unlimited space is available." The observations on our colony of *pipiens* undoubtedly deal with a different race than that of Richards' since the females required a blood meal for egg-laying (anautogenous). Concerning the mating behavior of an English autogenous race of *pipiens*, Tate and Vincent (1936, p. 136) noted that the males settled on resting females, forced their way beneath them and copulated while the females were at rest. These same writers observed that the behavior of the English anautogenous race of *pipiens* differed from the autogenous in that the males swarmed. When females entered the swarm they were pursued by males and pairing began in the air. Males of this strain did no attempt to pair with resting females, even when they lit near or upon them.

Pratt (1946) recorded *U. lowii* as copulating in the end-to-end position and also mentioned the fact that T. H. Aitken observed *U. cooki* Root mating end-to-end in a small lantern chimney. The two tarsal claws on each leg of the female *lowii* are small and simple (Figs. 42-44) and not suited for seizing the male. The tarsal claws on the prothoracic and metathoracic legs of the male (Figs. 39, 41) are somewhat similar to those of the female. However, his middle tarsus (Fig. 40) bears a single, large, curved tarsal claw which when flexed back reaches almost to the base of the last tarsal segment. Thus the males of *lowii* are similar to males of *quadrimaculatus* in that only one pair of legs is suited for seizing and holding the female; however, in *quadrimaculatus* it is the front pair of legs which is modified.

It is obvious from this discussion that there is still much to be learned about the mating positions and behavior of mosquitoes and that careful and detailed observations are needed to correct various conflicting reports found in the literature as well as to add new knowledge. If my hypothesis is true, namely, that the positions assumed by mosquitoes during copulation have evolved largely as a result of changes in the structure of the last tarsal segments of the front and middle legs of the male, then additional studies of

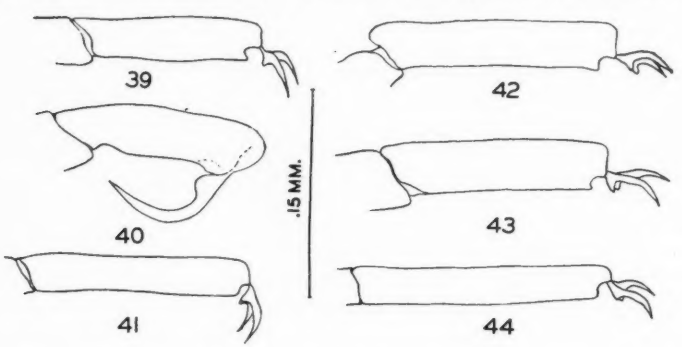
mosquito mating positions in various genera and species, correlated with male tarsal structure, should yield interesting results.

GENERAL DISCUSSION OF THE MATING BEHAVIOR OF MOSQUITOES

Until now this study has dealt mainly with the mating behavior of male *aegypti* using the female as the mating stimulus. The remainder of the study deals chiefly with the behavior of males to artificially produced sound stimuli. It is felt that the possible significance of some of the observations already noted should be discussed here rather than at the end of the section on sound.

Kahn et al. (1945) wrote of various sounds of mosquitoes as ". . . (a) mating calls, (b) calls warning of danger, (c) calls of anger and other sounds that are similarly functional." Beach (1945) rightfully criticized this interpretation and emphasized ". . . the dangers of interpreting the reactions of lower species in terms of psychological experiences characteristic of human beings." The present observations indicate that there is no specific sound produced by the female which can be interpreted as a "mating call," unless it be any sound she happens to make in flight, beginning several hours after emergence. Females which are placed in a cage with males and are made to fly continually so that the males copulate with them repeatedly, tend to resist the mating attempts of these males. Yet the sounds produced by the resisting females, during flight, continue to attract the males.

The male does not respond to a single fixed pitch but to frequencies which may vary over some range (see p. 326). Though possibly resulting in male *aegypti* attempting to copulate with other culicids, their response to a range of sound is "fortunate" for the species for it insures the "attractiveness" of different female *aegypti* which may produce sounds of different frequencies because of certain uncontrollable factors such as size, extent of distension of



Figs. 39-44. Last tarsal segments and tarsal claws of *Uranotaenia lowii*.—39. Male, prothoracic; 40. Male, mesothoracic; 41. Male, metathoracic; 42. Female, prothoracic; 43. Female, mesothoracic; 44. Female, metathoracic. (All drawings outlined with the aid of camera lucida.)

the abdomen with food, etc. If males responded only to a single fixed pitch, the number of attractive females would be diminished which in turn would reduce the number of impregnated females. There are several methods of biological isolating mechanisms which restrict random mating between species (Mayr, 1942, p. 247). The sound produced by flying female mosquitoes conceivably could be considered an isolating factor since it serves as the mating stimulus for the male. However, it is apparent from the behavior of *aegypti* that sound plays little part in sexual isolation since the male responds to a wide range of frequencies and will attempt to copulate with any mosquito the sound of which in flight falls within the range which will act as a mating stimulus. As far as *aegypti* is concerned sexual isolation must be accomplished in some other manner (mechanical, genetic, etc.). However, it is not impossible that some species may respond to a range of sound which is considerably narrower than that of *aegypti* and in these forms sound could play a part in sexual isolation. That *aegypti* males remain in a constant mating-state throughout their lifetime, once they have reached that state, and mate repeatedly with many females if given the opportunity, not only increases the chances for females to be fecundated but more than one female can be impregnated by a single male.

Both males and females tend to remain quiet for some time after emergence. Since copulation is initiated in flight, several possible benefits suggest themselves for this delay in flying. Undoubtedly this rest period gives the exoskeleton an opportunity to harden. Several investigators have determined the approximate age of *aegypti* at which fertilization occurs. Howard et al. (1912) and Putnam and Shannon (1934) claimed that both sexes mate within a few hours after emergence. Gutzevich (1931) stated that at 28° C., fertilization usually occurs 2 to 3 days after emergence. Seaton and Lumsden (1941) found that "Fertilization occurred with greater frequency as the age of the female mosquitoes increased, being exceptional on the first night and the rule on the third night after eclosion." From the present study it is apparent that the time of successful copulation depends upon three important factors. First, the female must become "attractive" and this occurs several hours after her emergence at which time the sound produced by her in flight reaches a pitch which will serve as a mating stimulus for the male. Though some females copulated 50 minutes after emergence after being induced to fly, it is probable that if the cage is left undisturbed copulations will occur later, since the females tend to rest for several hours after emerging. The flight of recently emerged females offers no benefit in mating since the pitch of the sound produced by these slow-flying mosquitoes is below the range which will stimulate sexually active males to copulate.

The second and third important factors in successful copulation concern the male. Not only must the genitalia rotate to about 180° but the antennae must also "mature" by the extension of the fibrillae to a condition where they are capable of perceiving the sound of the female in flight. It is probable that, unless disturbed, the male remains quiescent until this latter condition is reached. Some investigators have found that *aegypti* males often emerge before

females. Bacot (1916) noted that the early emerging males were usually a day quicker in their development than the females, and Gordon (1922) found that a much greater number of males than females emerged during the first few days of emergences. Shannon and Putnam (1934) in their laboratory study of *aegypti* observed that the average emergence period of females was 14 hours longer than that of males. The observations of all these workers are interesting in the light of the present study for they indicate that by the time the female begins to fly and becomes "attractive," the male's antennae have reached a state where the sound stimulus can be perceived and his genitalia has rotated sufficiently so that copulation can be successful (usually about 15 to 24 hours after emergence).

Recently emerged males are quickly seized by "old"[8] males because the sound produced by a young male in flight falls within the sound spectrum which stimulates a sexually active male to copulate. These older males cannot differentiate between the sex of very young males and several-hours-old females. Thus refraining from flight until some time after emergence avoids the attempted copulations of old males upon young ones; it was noted that when these attempted copulations were repeated often, one or more legs of the young male would be pulled off during the struggle. By the time a male begins to fly voluntarily, the sound he makes in flight is sufficiently high in pitch to be beyond the range which will stimulate old males and therefore he is unmolested.

Several workers (Lang, 1920; Howard et al., 1912; Hearle, 1926, and others) believed that, at least in those species which swarm, the female is attracted to the male. Concerning *C. pipiens*, Wesenberg-Lund (1921) wrote that females fly in a straight line into the swarm and "It was as if by some magic power the insects were forced to fly directly into the swarm of dancing males. Undoubtedly they were directed by the sound issuing from these males." Bates (1941) in his laboratory observations on the mating behavior of anopheline mosquitoes found that ". . . laboratory swarming males were not observed to exert any attraction on females, and mating occurred only when a female chanced into the swarm." In *aegypti* the male is the aggressor and is attracted by the female in flight. No evidence was found in the present studies to indicate that the female is attracted in any way by the male, although males may be flying about in a cage and a resting female may take to flight and be seized in mid-air. Some preliminary experiments indicate that the female does not react visibly (i.e. is attracted) to sounds that fall within the range made by males. Whether a female reaches a mating-state comparable to that in the male cannot be said; apparently it is impossible to separate any possible sexual activity from general activity since the female is passive and does not show any mating behavior similar to that of the male. Copulation will not occur until the female begins to fly and it is not known whether the stimulus which first induces the female to fly is sexual or nutritional. Whether or not the female is attracted by the sound of the male is still open to question.

8 The term "old" is used to designate males with their antennal fibrillae extended, and thereby capable of perceiving the sound of the female in flight.

After repeated matings, females become more and more reluctant to fly and if seized will also resist the male by pushing him away with her hind legs, or by jerking away her abdomen when he is about to make connection. This reluctance to fly and mate on the part of the female after repeated copulations may partly be due to fatigue (certainly the males are not fatigued after repeated copulations), or in a sense may actually be a form of "coyness." Richards (1927) suggested that repeated copulations would not only exhaust the individuals (i.e. insects in general) but also might expose the joined pairs to their enemies. He also noted that in some species coyness is only exhibited when the spermatheca is full.

It is not known how many times a female *aegypti* will copulate under normal conditions, but it is undoubtedly much less than the forced polyandry recorded in this experimental laboratory study. Only one copulation is necessary for fertile eggs to be laid (Hinds); normally only two of the three spermathecae are filled after one or two copulations, and this may be sufficient to induce coyness in female *aegypti*. Gibson (1945, p. 267) found that once the females of the chironomid *Spaniotoma minima* Meigen had copulated they did not rise again into the swarm of males, but remained resting on the floor of the cage. It is not known whether repeated copulations are in any way harmful to female *aegypti*. When a male seizes a female and she continues to fly about, other males are immediately attracted and will "mob" the pair trying to copulate. In many instances after a male seizes a female she quickly lands on the side of the cage and copulation is completed while at rest, thus preventing any interference from other males.

It would appear that with species that swarm in large numbers in the field, predators should be an important factor in survival. Howard et al. (1912, pp. 165, 171, 177, 179) give various records of dragonflies, wasps, birds, and bats feeding on adult mosquitoes. Gruhl (1927), quoted by Gibson (1945), believed that contrary to expectation, swarmers enjoyed a high degree of immunity from predators and he thought that this fact would act as a sustaining principle, tending to fix the habit, once it had been acquired. However, Banks (1919) noted dragonfles darting in and out of swarming anophelines, and Rao and Russell (1938) noted the appearance of bats simultaneously with swarms of *A. annularis*, which attacked mosquitoes and other insects swarming at the same time. Predators are of no imporatnce in the laboratory and though *Aedes aegypti* is chiefly a house mosquito with copulation often taking place indoors, several workers have found natural enemies of this mosquito. Bacot (1916) considered toads, lizards, spiders, ants, scorpions, and possibly young Mantidae to be destroyers of *aegypti*. Gordan (1922) concluded from experiments that both the gecko and a jumping spider readily destroyed *S. calopus* and *C. fatigans* and owing to their wide distribution were probably of some importance in limiting the numbers of mosquitoes occurring in human habitations. That the copulation of *aegypti* is rapid and the male releases the female as soon as the act is completed would decrease the chances of a joined pair being destroyed by other animals.

It is interesting to speculate upon the evolution of the epigamic mating

behavior of mosquitoes. The remarkable behavior of *Opifex* might well represent one of the most primitive recorded for mosquitoes, where apparently the sound produced by the female in flight plays no rôle in mating. As the behavior evolved to where it occurred in flight, the species swarmed. Gibson (1945, p. 293) in his review on swarming of Chironomidae suggested that swarming led ". . . to more easy meeting of the sexes through vision or possibly sound." In those species in which swarming occurs, the males generally congregate periodically, apparently the result of an internal rhythm. Gibson suggested that some form of nervous or hormonic change occurs at this time. At other times the males are in a sexually inactive state. From the epigamic behavior of the various species belonging to the *maculipennis* complex, swarming appears to be the older form of behavior, with this behavior apparently evolving away from the formation of swarms towards the individual dance, and finally to no dance at all. Thus the behavior of *A. atroparvus,* which may mate with or without swarming, led Cambournac and Hill (1940) to write that ". . . the evening dance, for *atroparvus,* has largely lost its functional character, although the form of the ritual is still preserved." Russell and Rao (1942) also found that swarming was not obligatory for the mating of *Anopheles culicifacies,* copulating pairs being seen in the absence of swarms; these matings took place at about the same time in the evening and always occurred in flight.

Knab (1906a) stated ". . . within the Culicidae, there is not only a marked diversity in the mode of swarming of different species, but that there are many forms that do not swarm at all. In the case of the almost exclusively house-inhabiting *Stegomyia calopus,* as Goeldi has shown, there are no such swarms as in *Culex* proper. The males congregate in little groups of 15 or 20, hovering over the corner of a cabinet or other prominent object, and pounce upon the females that come within range. I can add that copulation frequently takes place when there is no evidence of even such weak swarms." The present writer has also seen on several occasions pairs of *aegypti* flying freely in the laboratory, joined in the air, with no other mosquitoes flying about. According to Bates (1941, p. 169) the mating of *aegypti* generally is preceded by a sexual flight on the part of the male. However, the present study shows that this flight is not obligatory (in cages 11″ x 11″ x 15″) or a necessary prerequisite for mating. It is possible that the behavior of male *aegypti* has evolved from a form which originally swarmed to that shown today. Gibson (1945, p. 292) suggested that a change of habit, like the frequenting of small enclosures by a large number of individuals of the same species may result in the dance becoming ". . . divorced from the actual mating as a prelude to its total suppression."

Finally with regard to the attitudes assumed during copulation it is suggested that the modified linear position (Fig. 36) and the linear position (Fig. 38) have resulted from changes or modifications in the last tarsal segments and claws of the male's front and middle legs.

The Behavior of Male aegypti to Sound

HISTORICAL

It has long been known that mosquitoes react to sounds. Child (1894), Ross (1901), Osten Sacken (1901), Laurence (1902), Knab (1907b), Cambournac and Hill (1940), and Bates (1941) observed that various sounds altered the behavior of mosquito swarms. These sounds, which included the voice, whistle of a locomotive, sound of oars on water, a pistol shot, or the sounds from musical instruments, would attract or throw the swarms into some confusion. In most of these instances the swarms were males, although not all of the observers indicated which sex was involved. Nuttall and Shipley (1902) quoted Grassi as believing that persons engaged in conversation were more liable to be bitten by *Anopheles* than when silent; however this was no proof that the female was attracted by the sound of the voice.

Sir Hiram Maxim (1901) while erecting an electric lighting system in New York in 1878 observed that one of the dynamos used gave off a note which was practically constant and male mosquitoes attempted to enter the box from which the sound was being emitted. No female mosquitoes were attracted. Maxim suggested that the antennae were auditory organs and the males mistook the sound from the dynamo as the buzzing of the female. He also noted that striking a tuning fork, which closely resembled the sound of the female, would cause a male mosquito within 20 feet away to ". . . turn about, face the music, and erect the two little feathers on his head. . . ."

Mr. A. De P. Weaver, an electrical engineer, recorded observations similar to Maxim's and informed Howard (1901, p. 15) that ". . . while engaged in some experiments in harmonic telegraphy, in which a musical note of a certain pitch was produced by electrical means, he was amazed to find that when the note was raised to a certain number of vibrations per second, all mosquitoes, not only in the room where the apparatus was, but also from other parts and from outside, would congregate near the apparatus and would be precipitated from the air with astonishing force, striking their bodies against the apparatus."

Kahn and his co-workers (1945) made the interesting observation that ". . . when the call of a female is transmitted to two or three males under the circumscribed space of a small test-tube, it has been observed under the microscope that the antennae and hypopygium of the male will turn toward the direction from whence the sound is being transmitted." This "turning" of the hypopygium was apparently the first observation that the male is not merely attracted by certain sounds but actually shows a mating response to these sound frequencies.

THE MATING RESPONSE TO SOUND

The behavior of male *aegypti* to various sounds was studied with both tuning forks and an audio oscillator.[9] The male mating response, to sounds of different frequencies, can be separated into at least four distinct behavior patterns.

9 Model 200-A, Hewlett-Packard Co., Palo Alto, Calif.

Flight may be considered the first response to proper sound frequencies and can be demonstrated in the following manner. A cage full of virgin males several days old is placed about a foot or more away from a loud speaker[10] connected to the audio oscillator. The frequency dial of the oscillator is adjusted to produce a sound of 450 cycles with the amplifier dial reading zero. The oscillator is turned on. When all the males in the cage come to rest, the amplifier dial is turned momentarily so that the sound becomes audible. Many of the resting males will take to flight. By alternately making the sound audible and then subliminal, the majority of males will be induced to fly. A vibrating tuning fork (480 vps, etc.) when moved quickly along the outside of a cage containing males, also induces many individuals to fly.

The second response to sound is *attraction* to the source of the stimulus. When a cage containing many virgin male *aegypti* is agitated to induce flight. and a 480 tuning fork is struck and held near the outside, the males are attracted in large numbers to the sound source (Figs. 45-46). Often in their "frenzy" they seize one another and attempt to copulate. When the tuning fork is struck inside the cage and held among the flying males, they are attracted to the vibrating prongs of the fork. Males also follow the sounding tuning fork when it is waved about, indicating that the mosquitoes are responding to aerial vibrations and not to the vibrations of a substrate on which they might be resting. Males do not follow a moving, non-vibrating tuning fork.

Once the males are attracted they seize the cloth of the cage near the sounding fork, using their front and middle legs. At the same time their wings continue to vibrate and as they cling to the cloth their bodies shake violently; superficially it appears as though the males are being shaken by the sound vibrations. This third behavior is comparable to seizing the female in flight and therefore will be called the *seizing response*. It is possible that the rapid vibration of the male wings after seizing the female, lessens the burden of the latter and helps to maintain the pair in flight. If the tuning fork is struck near resting males or if the oscillator is used so that the sound emanating from the speaker is diffused, the seizing response sometimes is given from the resting position without the initial flight.

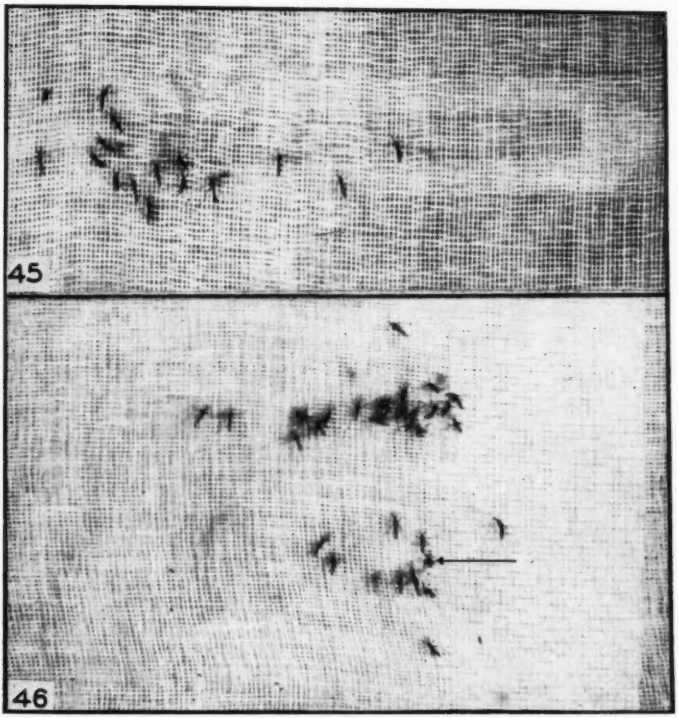
The fourth behavior pattern consists in flexing the abdomen ventrad so that the genitalia touch the cloth of the cage. This usually is done after the wings come to rest though some individuals flex the abdomen while the wings are still vibrating. This last response apparently is the one referred to by Kahn et al. as "turning" and corresponds to the final stage in copulation when the male makes connection with the female. The flexing of the abdomen will be referred to as the *clasping response*. Whether or not sperm is ejaculated during a clasping response evoked by mechanically produced sound was not investigated.

The seizing response can be elicited without the follow-up clasping response

10 Utah 3PW1; Utah-Radio Products Co., Chicago; 3½" permanent magnet speaker; 2½ watts.

and the clasping response sometimes is induced without the preliminary seizing response. When males fly to the source of sound the seizing response almost invariably is given. If males respond from a resting position without first taking to flight, either the seizing, clasping, or both responses are given. After repeated or continuous exposure to sound, the clasping response may be given without the initial seizing response. After a seizing response is evoked, the abdomen may be flexed ventrad and back several times so that more than one clasping response is elicited by the sound of a single tone, even after the sound is discontinued.

A typical mating response (i.e. attraction, seizing, and clasping) can be elicited from virgin males by the proper sound of the voice. Laurence (1902)



Figs. 45, 46. Attraction of male *Aedes aegypti* to the sound of a tuning fork (480 vps).—45. The outline of the tuning fork can be seen through the cloth side of the cage. Practically all of the males are shown giving the seizing response (vibrating the wings while seizing the cloth with the front and middle legs); 46. The arrow points to a male giving the clasping response (flexing the abdomen ventrad). (Photograph by E. R. Willis.)

attracted mosquitoes by the sound of the voice and stated, ". . . as far as my memory serves me, the sound that was most in favour was a continuous 'oo' pitched in a medium key, and then suddenly changed to a lower 'ah,' drawn out in the same way. Most vivid is the sudden rise in the volume of their noise, and the equally sudden attack on the face as they promptly answered the invitation."

OTHER REACTIONS TO SOUND

Male *aegypti* not only give a mating response to sound but also react to certain frequencies by (1) rubbing the antennae with the front legs, (2) rubbing the hind legs together, (3) rubbing the abdomen or wings with the hind legs, (4) jerking the body or hind legs slightly, (5) sudden flight, (6) suddenly coming to rest, (7) immobility, or (8) continuous activity (flying or walking). In general these are considered by Warden et al. (1940, p. 642) as the most primitive forms of insect behavior manifestations to sound and are grouped under the general term "shock-reactions." The present paper deals chiefly with the mating response of *aegypti* males, induced by various sound frequencies.

TUNING FORK EXPERIMENTS

The following available tuning forks were used to determine which sounds would induce the mating response: 256C, 288D, 320E, 341.3F, 384G, 426.7A, 480B, 512C, 1024C, 2048C, 4096C.[11] Either the seizing or clasping response, or both, were considered criteria for mating behavior.

Mosquitoes were exposed to the forks for a few seconds, or until the sound died out. Tests showed that the males respond to the sounds of forks ranging from 256 to 512 cycles but remain indifferent to the sounds of any of the above forks with frequencies greater than 512 vibrations per second. When positive, the response is usually instantaneous. When the response is negative, the male fails to respond as long as the sound is audible. Fifty-five males, more than 24 hours old, were placed (several at one time) in an 11″ x 11″ x 15″ cage and the different forks starting with the lowest (256) were struck and held on the outside of the cage near the resting mosquitoes. If the response was positive, the male was removed from the cage and was not subjected to any of the higher frequency forks, since in general males responded to all the sounds above the lowest frequency which stimulated them, up to 512 vps. If the response was negative, the next higher fork was used until a mating response was elicited. The 512 and 480 forks induced an immediate mating response, but since all the males tested responded to frequencies below 512 and 480, these two forks were not used in these tests. The results with the 55 males were as follows: 7 responded to 256, 5 to 288, 22 to 320, 12 to 341.3, 8 to 384, and 1 to 426.

Most males responded to frequencies between 320 and 512 vps, often being attracted from some distance away and following the fork when it was moved.

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11 The 1024 and 2048 forks were obtained from Central Scientific Co.; all others were made by Riverside Laboratories, Geneva, Ill.

Striking the forks lightly produced an immediate response from the insects. Males were not so readily attracted to the sounds of the 256 and 288 forks as they were to the others. Later, 100 and 128C forks were obtained, and these induced a mating response from virgin males several days old.

THE BEHAVIOR OF MALES SUBJECTED TO AN AIR CURRENT AND SOUND SIMULTANEOUSLY

Minnich (1925) found that a continuous air current inhibited the response of *Vanessa* larvae to sound. He explained this behavior as being due to one form of stimulation inhibiting the response to another form of stimulation.

When a cage full of male *aegypti* was placed directly in front of the breeze of an electric fan, the mosquitoes quickly came to rest on the sides and floor of the cage. As soon as the fan was shut off and the speed of the air current decreased the mosquitoes took to flight. A cage of males was placed a few feet in front of a fan and the speed of the current of air passing through the cage was measured with an anemometer. Two air speeds were used; one with the air passing through the front of the cage, nearest the fan, at a rate of about 2.8 miles per hour and through the back of the cage at about 2.3 mph; the other with the air passing through the front at about 3.5 mph and through the back of the cage at about 2.6 mph. The loud speaker connected to the audio oscillator was placed in the cage and various frequencies (400-600 cycles) were sounded after the fan was turned on and the males had come to rest (tuning forks were also used). Males continued to give both the seizing and clasping responses in spite of the breeze and flew to the source of sound. However, there was not the great attraction to the tuning fork that could be obtained when no current of air from a fan was blowing through the cage. When male *aegypti* were exposed to sound and an air current simultaneously, the mating response elicited by a tuning fork or sounds from an oscillator-speaker was stronger than the response to cling to the cage (remain at rest) when stimulated by an air current.

It should be pointed out, however, that Minnich's observations are not quite comparable to the present experiments on *aegypti*. With regard to *Vanessa* the air current caused the legs to cling to the substrata while the effect of sound induced the same larva to release its leg hold from the substrata. In this case the response to air current and sound were directly opposable. However, with *aegypti* the air current caused the males to come to rest and though some males flew to the source of sound (tuning fork) to give the mating response in spite of the air current, many gave a seizing or clasping response from the resting position without taking to flight. Since *aegypti* males can give a mating response from the resting position, the behavior responses to an air current and sound are not necessarily opposable.

THE MECHANISM OF AUDITORY PERCEPTION

Although no experimental evidence concerning the function and mode of action of Johnston's organ in the Culicidae has been obtained, the organ has

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generally been considered one of "hearing."[12] As early as 1855, Johnston concluded from morphological evidence that the pedicel of the antenna of *Culex* was auditory in function. Later Mayer (1874) observed the sympathetic vibration of the male antennal fibrillae to the sounds of various tuning forks, and he concluded that Johnston was correct in believing that this structure detects the sound produced by the flying female. According to Eltringham (1933, p. 106) the sensory function of Johnston's organ was quite obscure and still open to question. Warden et al. (1935, p. 315) wrote ". . . there is very little evidence to support the contention that the organ of Johnston is a sound receptor. Its wide distribution and its constant position in the second antennal segment suggest that it is a device for registering the movements of the flagellum of the antenna." Wigglesworth (1939, p. 136) stated, "Judging from its structure, Johnston's organ must be stimulated by movements of the flagellum of the antenna at its base." The lack of experimental evidence concerning the function and mode of action of this organ was summed up by Pumphrey (1940, p. 129) who wrote, ". . . , the name 'chordotonal organ' has come to be construed in a structural rather than a functional sense. Nevertheless, the presence of such an organ (Johnston's organ) in the base of the antennae of most insects has been taken to imply an auditory function for these appendages. The experimental evidence for such a view is very meagre. The observations of Mayer quoted in the Introduction do not necessarily imply that Johnston's organ is involved, for the long hairs on the antennae of male gnats are probably directly innervated and may respond to sound in a way similar to other long sensory hairs. From the work of Hollick (communication to the Society for Experimental Biology, 1939) it appears that in cycloraphous Diptera Johnston's organ responds to displacement of the antenna by air currents and plays an important part in flight in mediating the reflex adjustment of the wing-beat to the appropriate form for a given air velocity. According to Eggers (1926a), the same organ in *Gyrinus* responds to changes in curvature of the water surface. Johnston's organ is, therefore, obviously rather a labile structure and the possibility of its mediating sound reception in some insects can by no means be ruled out. The subject needs further investigation."

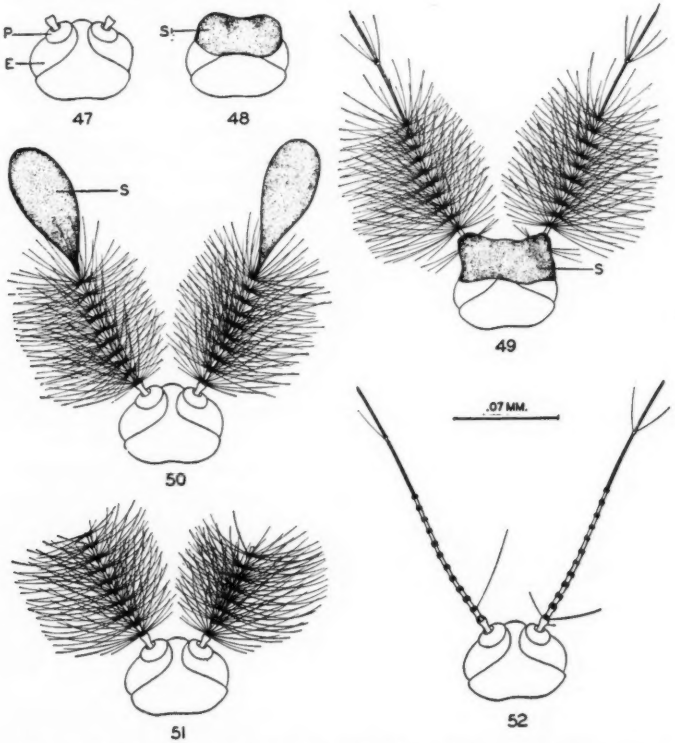
There are essentially two methods of studying hearing in insects. One is to observe the behavior of insects to various mechanically-produced sounds or the sounds made by other individuals of the species studied (Turner, 1914; Turner and Schwarz, 1914; Minnich, 1925, 1936, 1937; Abbot, 1927; Baier, 1930, and many others). The second method is to record action currents in auditory nerves in response to acoustical stimulation (Wever, 1935; Wever and Bray, 1933; Pumphrey and Rawdon-Smith, 1936a, b, c, 1937). The following experiments on the behavior of male *aegypti* towards females and the sounds of tuning forks were performed to test the validity of the hypotheses concerning the function and mode of action of Johnston's organ. During the tests, when-

12 The term "hearing" is used in the broad sense to indicate a response to vibratory stimuli by the aid of a phonoreceptor.

ever the males were exposed to females, the cage containing the mosquitoes was disturbed to induce the insects to fly.

Experiment 1.—To determine the behavior of antennectomized males to the sounds of tuning forks.

It has been shown earlier (see p. 293) that males which had their flagellar antennal segments removed close to the pedicelli were indifferent to



Figs. 47-52. Heads of male *aegypti* showing the various antennal operations performed to determine the function and mode of action of Johnston's organ.—47. Flagellar segments removed leaving small portions of the third segments extending beyond the pedicelli; 48. Flagellar segments removed and the pedicelli, remains of the third segments, and parts of the eyes covered with shellac; 49. Shellac placed at the base of the antennae covering the pedicelli, segments three and four, and parts of the eyes; 50. Tips of both antennae weighted with drops of shellac; 51. Weighted portions of the antennae in Fig. 50 amputated; 52. Practically all of the antennal fibrillae removed leaving the antennal shafts nearly bare.

Abbreviations: E—eye; P—pedicel; S—shellac.

females. The following experiment was performed to determine if similarly operated males would be indifferent to the sounds of tuning forks.

The flagellar segments of both antennae were removed, close to the pedicelli, from 10 males; only small stumps of the first flagellar segments and no fibrillae, remained (Fig. 47). Exposed 14 hours after the operation these males were activated (shock-reactions) by the sounds of the 480 and 512 tuning forks. Only one male gave a mating response; apparently the others, by the fact that they took to flight, were able to perceive the sounds. The experiment was repeated using 18 newly emerged males. These were exposed to the sound of the 480 fork, 42 hours later. None gave the mating response, but generally were activated to flight. All of these males had either the first or part of the first flagellar segments protruding beyond the margins of the pedicelli, with no fibrillae remaining.

Fifteen males had their flagellar segments removed and the remaining stumps and pedicelli covered with shellac (Fig. 48); parts of the eyes were also unavoidably covered. Tested 20, 46, and 54 hours after the operation, they remained completely indifferent to the sounds of the 480 and 512 forks.

Conclusion. Males which have their antennae eliminated by removal of the flagellar segments and covering of the remains with shellac fail to give a mating response to sounds of tuning forks.

Experiment 2.—To determine the behavior of males to females and tuning fork sounds after shellac was placed at the base of the antennae.

A small drop of shellac was placed, by means of a micro-pipette, at the base of the antennae (Fig. 49) of each of 20 non-virgin 7-day-old males. This operation cemented the first flagellar segments to the pedicelli and prevented the shafts from vibrating at their bases. The remainder of the segments and their attached fibrillae were free to vibrate. The males were exposed to the sound of a 480 tuning fork 12 hours later. Seventeen males were indifferent to sound; three males gave a mating response. These last 3 males were examined under a binocular microscope and each was found to have the hollow of one pedicel incompletely filled with shellac, so that apparently one shaft was capable of vibrating at its base.

The experiment was repeated using 20, 3-day-old non-virgin males. When exposed 4 and 17 hours later, they were all indifferent to the sound of the 480 fork. These males were also indifferent to 50 females introduced into the cage 4 hours after the application of shellac. All the males used in these experiments gave a mating response to the tuning fork sound prior to the application of shellac.

Conclusions. Males are indifferent to flying females and to the sounds of a tuning fork after the antennal shafts are prevented from moving at their bases.

Experiment 3.—To determine the behavior of males to females and tuning fork sounds after weighting the tips of the antennae with shellac.

Twenty-five, 3-day-old non-virgin males had one of their antennae weighted with a drop of shellac placed at the tip of the flagellum. These males were exposed to the sounds of a 480 fork, 19 and 28 hours later. Twenty-two males gave a mating response; three failed to respond. These males appeared to give a less vigorous response than normal individuals.

A small blob of shellac was placed on the tips, or apical third or half, of both antennae (Fig. 50) of 52, 3-day-old non-virgin males. The fibrillae not covered by shellac remained extended and the first flagellar segments were free at their bases. These males were exposed to the vibrations of the 480 fork at 12, 19, and 24 hours after the application of shellac. Only 2 males gave a mating response and one was found to have only a small amount of shellac (compared with the other antenna) on one of the antennae; the other male had 2 large drops of shellac at the tips of both antennae.

At the 12 and 19 hour trials, 27 of the males were removed, and the portions of the antennae bearing the shellac were amputated (Fig. 51). The number of remaining antennal segments varied from 5 to 9. Exposed to the 480 tuning fork, one-half to 5 hours after removal of the weighted portions, 25 males gave a mating response; one failed to respond and one had died.

After the 24-hour trial, 20 of the males having weighted antennae, and which failed to respond to sound, were placed in a cage with 5 females for 15 minutes. The males were completely indifferent to the females. The males were then removed and the shellacked portions of the antennae were amputated. Three hours later these same males, lacking the weighted parts of the antennae, were again exposed to 5 females, at which time they were attracted and attempted to copulate.

Conclusions. Males which have the tips of their antennae weighted with shellac are indifferent to females and sound. Males respond to females and sound, after the weighted portions of the antennae are amputated. Weighting the tips of the antennae apparently prevents the shafts from moving at their bases.

Experiment 4.—To determine the behavior of males to females and tuning fork sounds after removal of the antennal fibrillae.

Five males which had practically all of their antennal fibrillae removed with a camel's hair brush, leaving the shafts nearly bare (Fig. 52), were readily activated by the 480 and 512 forks and usually gave the seizing response when tested 19 and 45 hours after the operation. When the fork was sounded inside the cage and held near the males, they would fly to the vibrating prongs.

The experiment was repeated with 22 males, less than one-day-old. These were exposed to the 480 tuning fork at 39, 43, 50 and 66 hours after removal of the fibrillae. Seventeen gave a mating response. A seizing response was usually given, though some individuals also gave a clasping response. Forty-nine hours after the start of the experiment, 12 of the males which had given

a positive response to sound were placed in a cage containing 40 females. During the 15-minute trial, only 3 attempts to copulate were observed, one of which was successful. The male which had succeeded in copulating was examined under a binocular microscope and was found to have several antennal fibrillae remaining on the first and second flagellar segments.

Conclusions. Males respond to the sounds of a tuning fork after removal of practically all of the antennal fibrillae. Apparently the sympathetic vibration of the antennal shaft alone, to intense sound, stimulated the sense cells in Johnston's organ. The comparative indifference of these males to females may be explained as being due to the greater intensity of a tuning fork sound as compared to that produced by the female in flight. The antennal fibrillae may possibly serve as an "amplifier" in the sense that they are set into vibration by the less intense sound of the flying female. Lutz (1924, pp. 336-337), commenting on Mayer's tuning fork experiments, stated that the intensity of the sound of a female ". . . especially at a distance great enough to make it a desirable factor in guiding the male, is so slight that the sound from a tuning fork on a resonant box placed near and turned toward the antenna would be like that of an artillery battle in comparison."

Discussion.—Male *aegypti* show a definite attraction to certain sounds, and Wever's statement (Howard, 1901) that mosquitoes (species not indicated) would be "precipitated" against an apparatus producing certain sounds is quite applicable to the behavior of *aegypti* males to the sounds of various tuning forks. The mosquitoes are so drawn to the fork that the response reminds one of the inevitable attraction of iron filings to a magnet. The path taken by the flying male appears to be more or less a straight line; the type of orientation would possibly fit Fraenkel and Gunn's (1940) description of tropotaxis whose essential features are ". . . simultaneous comparison of intensities on the two sides, and orientation according to the balance thus struck." Concerning the sympathetic vibration of the antennal hairs of *Culex* to the sounds of a tuning fork, Mayer (1874, p. 586) stated that "If a sonorous wave falls upon one of these fibrils so that its wave-front is at right angles to the fibril, and hence the direction of the pulses in the wave are in the direction of the fibril's length, the latter cannot be set in vibration; but if the vibrations in the wave are brought more and more to bear athwart the fibril it will vibrate with amplitudes increasing until it reaches its maximum swing of co-vibration, when the wave-front is parallel to its length and therefore the direction of the impulses on the wave are at right angles to the fibril." From his observations he concluded that the sound of the female vibrated the fibrillae of one antenna more forcibly than those of the other and the mosquito turned his body in the direction of that antenna the fibrils of which were most affected. When the male brought the vibrations of each antenna to equality of intensity, his body faced in the direction of the radiation of the sound, and he directed his flight accordingly. Mayer further concluded that the male could guide himself to within 5 degrees of the direction of the female. Mayer's hypothetical explanation, and Johnston's before him, was essentially a tropotactic one. However, in the present experiments, it was found that males

lacking one antenna would fly to the vibrating prongs of a tuning fork, when the fork was struck and held inside the cage among the flying insects. These unilaterally flagellectomized males did not show continuos turning movements (circus movements, Fraenkel and Gunn, 1940, p. 77) which are supposedly characteristic of tropotactic responses. However, it will be recalled that the ability of the male to seize a female in flight is greatly reduced by the removal of all the flagellar segments from one of his antennae (see p. 293).

Tropotactic responses usually are associated with the term phototaxis (Fraenkel and Gunn, 1940). Snodgrass (1928) used the term phonotropism for the reaction of insects to sound. In keeping with more recent terminology, phonotaxis is hereby used to indicate the influence of certain sound frequencies on the movement of males of *aegypti*.

Fulton (1928, p. 553) stated that Mayer's tuning fork experiments did ". . . not actually prove that mosquitoes hear with their antennae any more than it could be proved that a piano hears when its wires are set into sympathetic vibration by sound waves. The mosquito gave no indication that it perceived any sensation of sound." Warden et al. (1935, p. 313) also stated that Mayer's work was ". . . of small value, unless supported by evidence from behavior." The foregoing behavior experiments, summarized in Table II, support Mayer's hypothesis and confirm the hypothesis that Johnston's organ is aural in function, an assumption previously based on morphological evidence. The experiments also prove that the males perceive vibratory stimuli as had been hypothesized in the literature. That is, the antennal fibrillae or whorls of hairs, vibrating in response to certain sound frequencies, cause the antennal shaft to vibrate. The movement of the flagellum of the antenna on its base stimulates the sense cells attached to sclerotized processes of the circular plate at the base of the first flagellar segment (Fig. 6) and the resulting impulse travels along the antennal nerve to the brain. The antennal fibrillae apparently are not innervated directly as suggested by Pumphrey (1940) since males generally fail to respond to sound when the majority of fibrillae are free to vibrate but movement of the antennal shaft at its base is prevented (see Experiments 2 and 3, pp. 318-319).

Howard et al. (1912, p. 35) stated that "Our own observations of the habits of mosquitoes lead us to doubt that the antennae, if auditory at all, are so in their primary function. The foregoing hypotheses are founded upon the assumption that the male seeks the female and is attracted by her song. In fact such is not the case. At least in those forms in which the antennae of the male are most highly specialized the males 'swarm' and the female seeks the male. In our experience the song of the female does not attract the male." It has been pointed out (see p. 308) that there is no experimental proof that the female is attracted by male swarms. The sound of female *aegypti* is the chief, if not the only, external stimulus that will induce mating behavior on the part of males, and when exposed to certain mechanical sound waves, these males will show a characteristic mating response which is comparable to the copulating behavior with the female. Although the female has a Johnston's organ, it is not as well developed as that of the male. The mode of attach-

ment of the sense cells to the base of the third segment (in *Culex* and *Corethra*, see Howard et al., 1912, Plate 1, Fig. 7) indicates that the structure would be much less efficient in responding to movements of the flagellum at the base. Female *aegypti* exposed to a wide range of sound frequencies from the audio oscillator-speaker showed no unusual behavior other than some shock-reactions.

TABLE II.—Summary of the Behavior of *Aedes aegypti* Males, under Various Experimental Conditions, Towards Female *aegypti* and to the Sound of Tuning Forks (480 and 512 vps).

Type of antennal operation	*Behavior*	
	To female	*To tuning fork*
1. Normal male (Fig. 13)	Seized female in flight and copulated	Gave either a seizing or clasping response or both
2. Flagellar segments removed close to the pedicelli, or below the apex of the first flagellar segments, leaving no fibrillae (Fig. 47)	Ignored the females	Rarely gave a mating response but some were activated to flight
3. Complete antennectomy; flagellar segments removed and pedicelli and remaining stumps of the first flagellar segments covered with shellac (Fig. 48)	Not exposed, since males in 2, above, ignored females	Unresponsive
4. Shellac placed at the base of the antennae, completely covering the basal flagellar segments and pedicelli; remainder of the shafts and fibrillae were free to vibrate (Fig. 49)	Ignored the females	Unresponsive
5. a) Tip of antenna weighted with drop of shellac	Not exposed to females	Generally gave a mating response
b) Tips or about upper third of both antennae weighted with shellac (Fig. 50)	Ignored the females	Generally did not respond (may depend upon weight or amount of shellac applied)
c) Weighted portions of antennae in b), above, amputated (Fig. 51)	Attracted to females and copulated	Gave a mating response
6. Practically all of the antennal fibrillae removed, leaving the antennal shafts nearly bare (Fig. 52)	Generally ignored the females	Usually gave a mating response; some males show directional response, flying to the vibrating prongs of the fork

Child (1894) believed that the function of Johnston's organ was originally the perception of tactile stimuli and after the structure became more highly developed it also served for the perception of sound waves. Howard et al. (1912) did not deny that the antennae were also in a rudimentary way auditory in function. They claimed that though ". . . mosquitoes react to sound-waves, it is by no means clear that they can perceive them as such. If the sound-impressions are received through the antennae, as the organization of these appendages seems to indicate, it is still to be doubted that there is a differentiation of the impressions produced by sound-waves and other stimuli, such as a current of air, or between sounds of different pitch." Mosquitoes orient to air currents, and *aegypti* males will come to rest quickly in a cage when exposed to the current from an electric fan. Undoubtedly the antennae are deflected by gross air movements but it cannot be said for certain that the males come to rest as a result of the antennae being set into motion by the air current. Although it has been assumed that mosquitoes orient themselves to air currents by means of their antennae, Kennedy (1939) found evidence to indicate that in female *aegypti* vision plays an important rôle in up wind orientation. However, male *aegypti* respond to certain sound frequencies by giving a mating response, yet these same males may react to other frequencies by giving a shock-reaction (i.e. jerking the body, rubbing the hind legs together, etc.). These differences in behavior imply some form of discrimination, and this may be accomplished by different numbers of sense cells of Johnston's organ being stimulated. Adrian (1928, p. 31) concerning nerve fiber impulses stated that ". . . the only way in which the message can be made to vary at all is by a variation in the total number of the impulses and in the frequency with which they occur." The deflection or movement of the flagellum of the antenna of *aegypti* males may vary in response to different sound frequencies and air currents, and this in turn could vary the type of response in the antennal nerve. Pumphrey and Rawdon-Smith (1936b) showed that the responses of the cercal nerve of the cockroach varied with the frequency of the auditory stimulus. The same authors (1936b, c) found that the cerci of the cockroach were very sensitive to movements of the air since action currents were recorded from the cercal nerve after exposing the cerci to a ". . . light puff of air." The action current recorded from the cercal nerve after stimulating the receptor by air movements was different from the impulses recorded after stimulating the organ with sound. In 1939, Pumphrey and Rawdon-Smith advanced a theory to explain "frequency discrimination" in insects possessing tympanic organs. Final proof that Johnston's organ of male *aegypti* is actually capable of distinguishing between an air current and sound, and between different sound frequencies, can be obtained by recording action currents from the antennal nerve after subjecting the antennae to these various stimuli.

AGE OF MALE AT FIRST MATING RESPONSE TO SOUND

It was shown earlier that male *aegypti* may attempt to copulate when their genitalia have rotated about 135 degrees. These attempts are generally unsuccessful because a rotation of about 180 degrees is necessary before the male can make connection with the female. The following experiment was per-

formed to determine how soon after emergence the male will show a mating response (either seizing, clasping, or both) to the sounds of tuning forks.

Ninety males isolated at emergence were exposed to sounds of tuning forks at various intervals; the forks were struck and held near the mosquitoes. Six males gave positive responses to the 288 and 320 forks as early as 5 hours and 20 minutes after emergence; 12 individuals responded between 5 hours and 20 minutes and 11 hours after emergence. Practically all of the others responded about 14 hours 40 minutes or later, to the 480 and 512 forks. The early responses were obtained from males the genitalia of which had rotated less than 90 degrees. Most of the males which responded to the sounds of the tuning forks about 15 hours or less after emergence did not have completely rotated genitalia. Also, many would not attempt to copulate when placed in a cage containing females. This response to the tuning fork and indifference to the female may be explained by the fact that the sound of the flying female is much less intense, and more transient than that of a tuning fork being sounded and held near the male.

Thus the mating-state or condition when the male will show a seizing or clasping response (mating behavior) may be reached long before he is either physically capable of copulating successfully because of incompletely rotated genitalia or before he will even attempt to copulate with females. If the eighth abdominal segment of the male is ligated with a fine thread, before little or no rotation has taken place, the genital segments are prevented from rotating. This was done to 6 males about 4 to 5 hours after emergence, and 15 hours later all gave mating responses to the 320 and 426.7 vps. tuning forks. The male mating response elicited by a female in flight apparently is correlated with or dependent upon the condition of his antennal fibrillae, rather than the rotation of his genitalia. In young males, less than 5 hours old, the antennal fibrillae cling together, are flattened against the shafts, and apparently are incapable of covibrating even to the intense sound of a tuning fork. It is possible that some internal change takes place or perhaps the antennal shafts harden; older males which had nearly all their fibrillae removed, leaving the shafts practically bare, gave a mating response to the sound of a tuning fork (see p. 320). As the male ages, the antennal fibrillae, beginning with the ones on the basal segments, become extended and thereby capable of being set into sympathetic vibration by sound stimuli. Though some males less than 15 hours old may respond to tuning fork sounds, they often ignore flying females presumably because the female sound is much less intense than the sound of a vibrating fork, and not enough of the male antennal fibrillae are extended to perceive the slight sound made by the female in flight.

It is generally accepted that sexual behavior apparently is uninfluenced by the gonads (Wigglesworth, 1939, p. 383). Bates (1939) found that the F_1 males of the *atroparvus* x *maculipennis* cross, even when rendered completely sterile because of atrophied gonads, formed in a swarm and attempted to mate when placed in a small cage. From this he concluded (1941, p. 170) that the sexual behavior pattern is probably ". . . not dependent on the functioning of the primary sexual organs, and that it may have a complex genetic basis, since

the characters of the F_1 seem to differ from the parental characters and may represent a blend."

PRELIMINARY AUDIO OSCILLATOR EXPERIMENTS TO DETERMINE THE RANGE OF SOUND FREQUENCIES WHICH WILL INDUCE THE MATING RESPONSE

The previous tuning fork experiments did not determine the limits of sound which will induce the mating response in male *aegypti*. Not enough forks were used and pure tones were not produced. A tuning fork gives a single pure tone only when struck gently at the proper place with a fairly soft hammer; otherwise overtones are produced, and these are not necessarily harmonic (Saunders, 1936, p. 286). No attempt in any of the tuning fork experiments was made to produce a pure tone, the forks usually being struck at their tips with a resulting very intense sound. To determine the upper and lower limits of sound frequencies which will induce the mating response, an audio oscillator was used, for with this instrument a wide range of sound frequencies could be explored and relatively pure tones were produced.

The following preliminary experiments were undertaken to give the writer some idea as to the lower and upper limits of sound frequencies which would induce the mating response in *aegypti* males. The audio oscillator was hooked up to the 3½" loud speaker. Two methods were used with these preliminary studies. With the first, the speaker, which was built into a small wooden box, was placed next to one side on the outside of a cage containing several hundred virgin males more than 24 hours old. The speaker was left stationary or moved back and forth from one end of the cage to the other. With this method the sound was not concentrated at one point but was somewhat diffused throughout the cage so that comparatively few males were attracted to the source; most of the individuals responded wherever they happened to be resting in the cage. With the second method, a glass tube 1¼" in diameter was fitted into the speaker and the whole box was wrapped in several layers of absorbent cotton so that the sound when emitted was more concentrated near the opening of the tube. The speaker was moved about and placed with the opening of the attached tube near the resting mosquitoes. With this method and the proper frequency there was some attraction, and males flew from a short distance seizing the cloth next to the opening of the tube. The attraction obtained with the speaker attached to the audio oscillator was never so marked as that obtained with tuning forks, this apparently being due to the fact that a tuning fork, in comparison to the speaker, produced a more or less concentrated point source of sound. The full range of the oscillator, from 35 to 35,000 cycles per second was explored, the frequencies being changed at few second intervals. It is not known whether the speaker used was able to produce the very high frequencies and it is doubtful that sounds above 5,000 cycles were of desirable purity and intensity. However, the males responded to rather low frequencies (about 150 to 750 cycles) so that the higher frequencies did not have to be tested. Several tests were run, first starting with the lowest frequency and working up, then starting with the highest and working down.

Starting with the lowest and working upwards, the lowest frequency which induced a mating response was 150 cycles. However, few mosquitoes gave a positive response to sounds between 150 and 300 cycles. When exposed to sounds whose frequencies ranged from 100 to 300 vibrations per second, many males showed the usual shock-reactions such as (1) a slight twitching of the abdomen, (2) cleaning the antennae with their front legs, (3) waving their hind legs up and down or rubbing them together, etc. A large number of males gave the mating response to frequencies between 350 and 650 cycles.

Starting with the highest frequencies and working downwards, the first sound to induce many males to respond was 650 cycles with some individuals responding to 750 and 700 vibrations per second.

After a group of mosquitoes was exposed to certain frequencies (i.e. 300-600) some individuals responded to lower frequencies which they ignored initially. Thus in one trial while no mating response was given to the first sounds of 200-250 cycles, some males later responded to these frequencies after reacting to higher frequencies. Also some individuals which failed to respond to 700-750 vibrations initially, responded to these when they were sounded a second time, after the males had been made to respond to frequencies between 600 and 400 cycles. Since there was a comparatively brief silent period between the soundings, it was possible that the response to one frequency was carried over to the sounding of another.

Conclusions. From these preliminary trials it is seen that the limits of sound frequencies which will induce the mating response in male *aegypti* are about 150 and 750 cycles. In the next section it will be shown that these limits are influenced by age and virginity or non-virginity of the males.

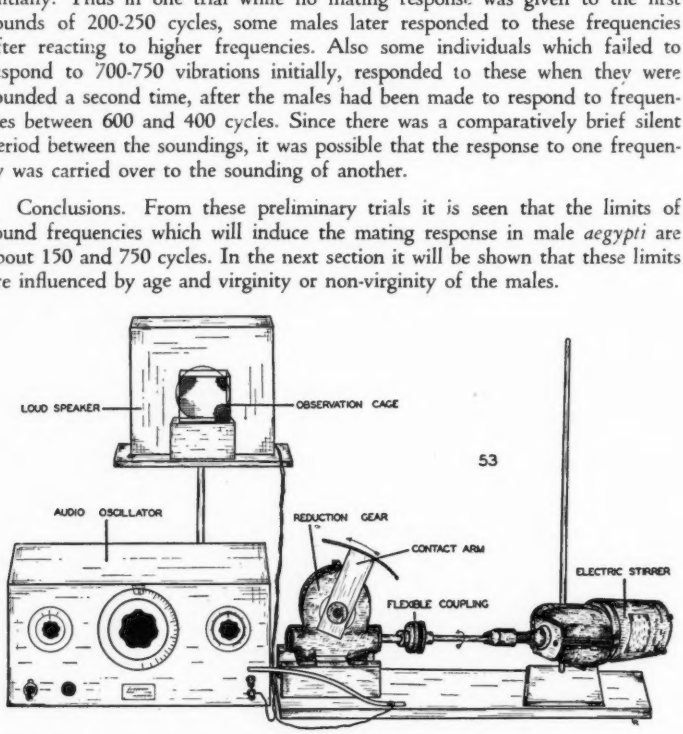


Fig. 53.—Apparatus used for determining the limits of sound frequencies which will induce the mating response in male *Aedes aegypti*.

DETERMINATION OF THE UPPER AND LOWER LIMITS OF SOUND FREQUENCIES WHICH WILL INDUCE THE MATING RESPONSE IN VIRGIN AND NON-VIRGIN MALES

Method.—The previous preliminary experiments indicated that the mating response could be elicited from male *aegypti* by sound frequencies ranging from 150 to 750 vibrations per second. It was also noted that there was some variation between different males in their response to this range of sound frequencies.

For the following experiments an apparatus was devised whereby mosquitoes could be exposed to known frequencies of sound from a speaker connected to an audio oscillator, sounded for definite lengths of time at fixed and regular intervals. The apparatus (Fig. 53) consisted of an electric stirrer connected by a flexible coupling to a reducing gear having an attached arm which when rotating would make and break the contact with the oscillator. By varying the speed of the stirrer, or using arms of different lengths on the reducing gear, frequencies could be sounded for desired lengths of time at desired intervals. Individual mosquitoes were placed in a small (3″ x 3″ x 1″) cloth, wire-framed cage with celluloid sides for observation. The cage was then placed up against the circular opening of the box containing the loud speaker and the oscillator was turned on. With the observation cage in this position the mosquitoes could be about ½ to 2 inches away from the speaker at the moment of exposure to the sound. The electric stirrer produced an audible hum in operation but males apparently did not react in any way to this sound. The behavior of the mosquitoes to the various frequencies was observed and noted; only the seizing and clasping response, or both if given, were considered criteria for the mating response. All other reactions to various frequencies, such as rubbing the antennae with the front legs, rubbing the hind legs together, rubbing the abdomen or wings with the hind legs, a slight jerking motion of the abdomen, suddenly coming to rest, immobility or continuous activity, although indicative of a response to sound, were not considered. The following tests determined only the limits for inducing the mating response and not the limits of hearing.

To determine the upper and lower sound limits for inducing the mating response, individual male *aegypti* were subjected to frequencies ranging from 100 to 800 vibrations per second. For the upper limit each male was exposed to frequencies starting with 800 cycles. Each frequency was sounded 3 times, each time for 3 seconds at 6-second intervals. If the male failed to respond during these 3 trials, the frequency was lowered 50 cycles, during the third, 6-second silent interval, to 750 cycles and so on until a positive response was obtained. After responding, the male was returned to the stock cage. If a male failed to respond by the time 350 cycles was reached, he was counted as not responding. The lower limit was determined in the same way; however, the frequencies used started with 100 cycles, proceeding upwards by 50 cycle increases until a positive response was obtained. If a male failed to respond by the time 600 cycles was reached, he was counted as not responding.

Usually from 1 to 3 males were in the observation cage at one time; the other males still to be tested were kept outside the room until exposed to the sounds of the oscillator-speaker. Males responded from all parts of the observation cage (facing or with their backs or sides to the speaker) though some individuals not responding to the initial sounding of a frequency would reorient themselves by flying or walking and respond to the same frequency when sounded a second or third time.

TABLE III.—Approximate Intensities of the Various Sound Frequencies when the Amplifier Dial of the Oscillator Read 100. These were the Frequencies and Intensities used to Determine the Upper and Lower Limits of Sound which would Induce the Mating Response in Virgin and Non-virgin Male *aegypti*.

Sound frequencies	*Intensities in decibels*
100	95.6
150	103.8
200	112.6
250	113.2
300	111.0
350	109.0
400	108.3
450	109.0
500	108.6
550	108.5
600	109.9
650	110.7
700	107.1
750	106.8
800	106.1

All the frequencies were sounded with the amplifier control of the oscillator set at 100, so that each frequency was produced at its fullest intensity. A sound level meter[13] was used to determine the intensities of the different frequencies used. The intensity readings expressed in decibels, shown in Table III, can only be considered approximations since readings were made in a comparatively small photographic dark room, where the mosquitoes were exposed to sound. Reverberations of the various sounds against the walls influenced the readings on the sound level meter. Even the position of the observer with respect to the microphone of the meter influenced the resulting intensity readings. The intensities of the different frequencies were determined in both the dark room and a larger laboratory room. In both tests the center of the microphone of the meter was 2½ inches away from the loud speaker. The intensity readings shown in Table III are based on an average of five readings for each frequency. Intensity readings taken in the larger laboratory room were slightly lower than those made inside the dark room where the mosquitoes were tested. Readings were also taken with the microphone 6 inches away from the loud speaker and the intensities registered were lower but

13 Type 759-A, General Radio Co., Cambridge, Mass.

followed the same general curve obtained when the readings in Table III were graphed. It can be seen that with the amplifier control of the oscillator reading 100, the different frequencies varied somewhat in intensity. Though the greatest intensities capable of being produced by the apparatus were used, males responded to sounds that were barely audible.

The upper and lower limits for 6 age groups of virgin and non-virgin mosquitoes were determined in the manner described. In some cases the same males were used for 2 or 3 age groups but because of the comparatively long interval between the groups, the exposure to sounds at one age did not influence the insect's behavior at a later age. The results obtained with the same males exposed to sounds at different periods in their lives were essentially similar to those obtained with same aged males exposed to sounds for the first time.

Determinations of the limits of virgin males were made from individuals kept isolated from females until exposed to sound. The results shown in Tables IV and VI are based on 100 positive responses for the upper and 100 positive responses for the lower sound frequency limits for each age group; thus the limits for all virgin age groups are based on a total of 1200 males giving a mating response. In the determination of non-virgin sound frequency limits, the males were kept with females until the former were exposed to sound. The cages containing these mosquitoes were usually agitated several times a day to insure repeated copulations. The age groups tested were the same as for virgin males. The results shown in Tables V and VII are based on 50 positive responses for the upper and 50 positive responses for the lower limits for each age group, giving a total of 600 responses for all non-virgin groups. The number of virgin and non-virgin males failing to respond is also shown in Tables IV-VII.

Since in determining the limits the sound frequencies were lowered or raised by 50 cycles, theoretically a male could have responded to a frequency anywhere between the last sound frequency which failed to induce a response and the frequency which stimulated the male to give a mating response. For example, males which failed to respond to 800 cycles but did respond to 750 could have responded to any frequency between 750 and 800 since these intermediate sounds were not used. For this reason the frequencies shown in Tables IV-VII and Figs. 54-57 are not the actual sounds used (see Table III) but represent the centers of the frequencies tested. Thus in the determination of the upper limits, males which did not respond to 800 but did respond to 750 were assumed to have responded to 775 cycles, the center point between 750 and 800; males which failed to respond to 800 and 750 but responded to 700 cycles were considered as responding to 725 cycles, etc. Individuals that responded to 800 were arbitrarily designated as responding to 825 cycles since in the following tests no males were exposed to sounds above 800 vibrations per second. In determining the lower limits, males which failed to respond to 100 cycles but did respond to 150 were assumed to have responded to 125 cycles, etc. Males which responded to 100 were arbitrarily designated as responding to 75 cycles, since 100 vibrations per second was used as a starting point.

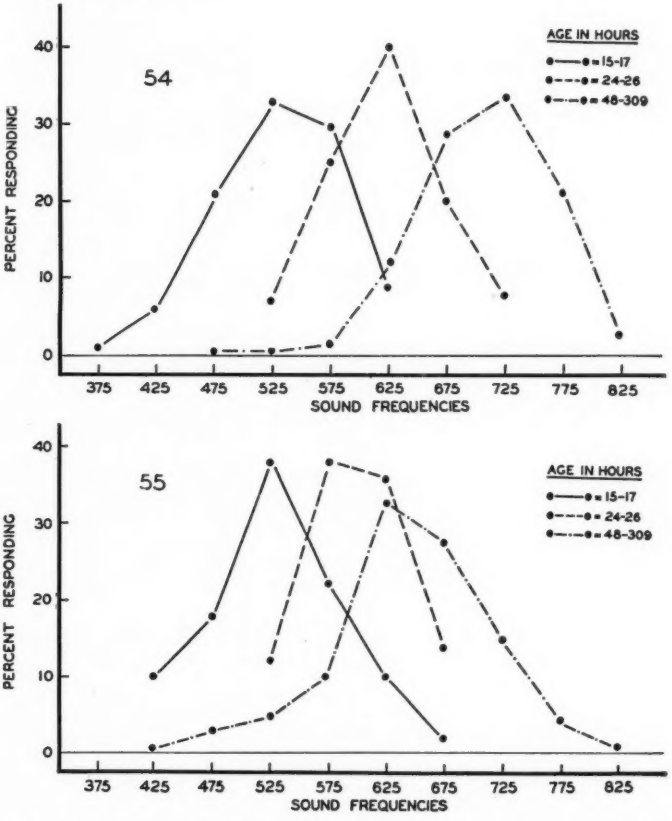
Upper sound frequency limits which will induce the mating response in virgin and non-virgin males.—Table IV shows that in each virgin age group a high percentage of males responded to certain frequencies, with fewer individuals responding to sounds above and below these frequencies. The mean frequency for the 15-17-hour-old males was 531 cycles, for the 24-26-hour group 623.5 cycles and for the 4 older groups, 709.5, 701.5, 721 and 702.5 cycles respectively. The differences between the mean limits of the 4 oldest groups were small as compared to the rise in mean upper limit between the 15-17 and 24-26 age groups, and the 24-26- and 48-50-hour groups so that the data from the 4 oldest groups were combined (Table IV, age group 48-309) to give 2 main shifts in the upper limit, with increase in age. This is clearly shown in Fig. 54. In the 15-17-hour group the peak of response occurs about 525 and 575 cycles, in the 24-26-hour group the peak is 625 cycles, while in the 48-309 group the peak is about 675 and 725 cycles. With increase in age the males respond to higher frequencies.

Table V shows the results obtained in the determination of the upper limits of sound frequencies which induced the mating response in non-virgin males. The results are comparable to those obtained with virgin males, and by combining the data of the 4 oldest groups we find, as with virgin males, that there are 2 main changes in the limits, clearly shown in Fig. 55. However, it is evident that the upper limit does not rise as high in non-virign males as it does in the virgin groups; the peak of response for the non-virgin 15-17 group is 525 cycles, for the 24-26 group about 575 and 625 cycles and for the 48-309 age group about 625 and 675 cycles (cf. Figs. 54 and 55).

Lower sound frequency limits which will induce the mating response in virgin and non-virgin males.—Table VI shows the results obtained for the determination of the lower limit of virgin males. Here, too, we find a shift in the lower limit with increase in age. By combining the groups whose mean frequency limits are comparatively slightly different, we find 2 main drops. These drops do not occur simultaneously with the rise in the upper limit, but take place between the 48-50- and 63-73-hour groups and between the 157-176 and 203-309 age groups. Figure 56 shows that except for the 203-309 age group, the drop in the lower limit is not so marked as the rise in the upper limit of virgin males (cf. Figs. 54 and 56). The curve for the lower limit does not move uniformly to the lower frequencies with increased age. The drop is apparently due to an increase in the number of individuals responding to frequencies below 275 cycles. Thus in the 15-50-hour-old males only 2.99% responded to frequencies below 275 cycles, 16.5% of the 63-176-hour-old males responded to frequencies below 275, while 59% of the 203-309 age group gave a mating response to frequencies below 275 vibrations per second. The greatest drop in the lower limit occurs in the oldest males tested.

Table VII gives the results obtained in the determination of the lower limit of non-virgin males. Here, too, there is a drop in the limit, with an increase in age, which occurs largely between the 48-50- and 63-73-hour groups and the 63-73 and 157-176 age groups. The change in sound limits for the combined groups is shown in Fig. 57, and it can be seen that the drop is partly due to a more or less uniform shift of the curve to the lower frequen-

cies and partly to a small increase in the number of males responding to frequencies below 275 cycles. Only 2.6% of the 15-50-hour-old males responded to frequencies below 275, 6% of the 63-73-hour-old males responded to frequencies below 275 cycles while 20% of the 157-309 age groups responded to sounds below 275 cycles. It is clear that the drop in the lower limit of the oldest non-virgin males tested is not as marked as that of virgin individuals (cf. Figs. 56 and 57).



Figs. 54, 55.—54. Graph showing the upper limits of sound frequencies which induced the mating response in three age groups of virgin *aegypti* males. The age group 48-309 represents the combined data of the four oldest groups tested, 48-50, 63-73, 157-176, and 203-309. (Data from Table IV); 55. Graph showing the upper limits of sound frequencies which induced the mating response in three age groups of non-virgin male *aegypti* (males kept with females until exposed to sound). The age group 48-309 represents the combined data of groups 48-50, 63-73, 157-176, and 203-309. (Data from Table V)

TABLE IV.—Upper Limits of Sound Frequencies which Induced the Mating Response in Six Different Age Groups of Virgin Male *aegypti*. (The Age Group 48-309 Represents the Combined Data of the 48-50, 63-73, 157-176, and 203-309 Age Groups. The Percentages given under the Combined Data Column are Based on 400 Positive Responses.)

Age of males (hours)	Number of males responding	Sound frequencies (vibrations/second)										Mean frequency	Total mosquitoes used	Number of males not responding
		375	425	475	525	575	625	675	725	775	825			
15 - 17		1	6	21	33	30	9	0	0	0	0	531.00	118	18
24 - 26					7	25	40	20	8	0	0	623.50	109	9
48 - 50						1	10	32	34	22	1	709.50	100	0
63 - 73				1	1	0	11	32	40	14	1	701.50	102	2
157-176						1	12	20	34	27	6	721.00	100	0
203-309						3	15	32	26	22	2	702.50	100	0
		Combined data												
48-309				1	1	5	48	116	134	85	10	708.62	402	2
				.25%	.25%	1.25%	12%	29%	33.5%	21.25%	2.5%			

TABLE V.—Upper limits of Sound Frequencies which Induced the Mating Response in Six Different Age Groups of Non-virgin Male *aegypti*. (Males Kept with Females until Exposed to Sound; Age Group 48-309 Represents the Combined Data of Groups 48-50, 63-73, 157-176, and 203-309; the Percentages of Age Group 48-309 are Based on 200 Positive Responses.)

Age of males (hours)	Number of males responding	Sound frequencies (vibrations/second)									Mean frequency	Total mosquitoes used	Number of males not responding
		425	475	525	575	625	675	725	775	825			
15-17		5	9	19	11	5	1	0	0	0	530	87	37
24-26				6	19	18	7	0	0	0	601	70	20
48-50				2	6	19	14	7	2	0	649	65	15
63-73		1	3	3	2	18	13	8	1	1	640	60	10
157-176				3	3	19	19	3	2	1	651	58	8
203-309			3	2	9	10	9	12	4	1	652	105	55
		Combined data											
48-309		1	6	10	20	66	55	30	9	3	648	288	88
		.5%	3%	5%	10%	33%	27.5%	15%	4.5%	1.5%			

TABLE VI.—Lower Limits of Sound Frequencies which Induced the Mating Response in Six Different Age Groups of Virgin Male *aegypti*. (The Age Group 15-50 Represents the Combined Data of the 15-17, 24-26, and 48-50 Age Groups and the Age Group 63-176 is the Combined Data of the 63-73, and 157-176 Age Groups. The Percentages Given under the Combined Data Column are Based on 300 Positive Responses in the 15-50 Age Group and 200 Positive Responses in the 63-176 Age Group.)

48-309	Number of males responding	1	6	10	20	66	55	30	9	3	648	288	88
		.5%	3%	5%	10%	33%	27.5%	15%	4.5%	1.5%			

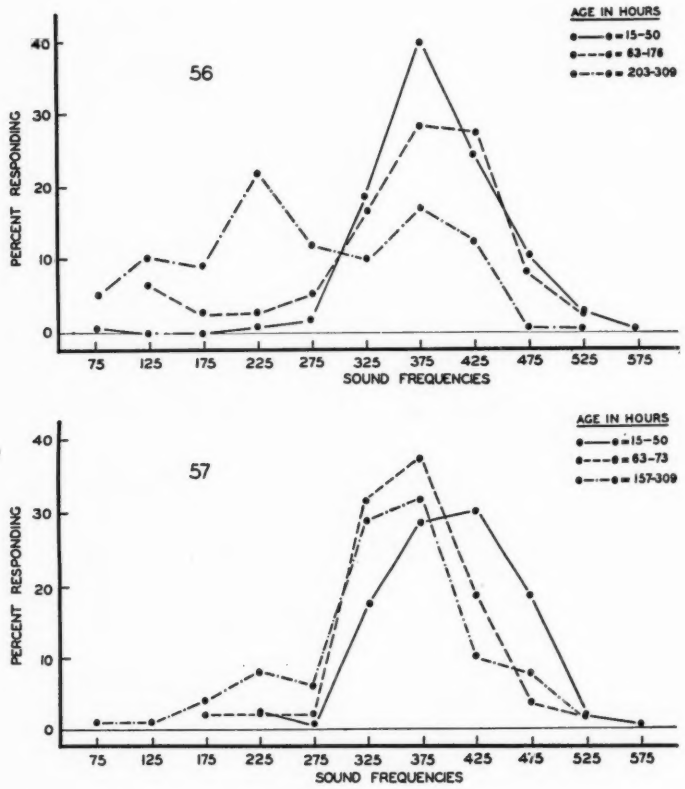
TABLE VI.—Lower Limits of Sound Frequencies which Induced the Mating Response in Six Different Age Groups of Virgin Male *aegypti*. (The Age Group 15-50 Represents the Combined Data of the 15-17, 24-26, and 48-50 Age Groups and the Age Group 63-176 is the Combined Data of the 63-73, and 157-176 Age Groups. The Percentages Given under the Combined Data Column are Based on 300 Positive Responses in the 15-50 Age Group and 200 Positive Responses in the 63-176 Age Group.)

Age of males (hours)		Sound frequencies (vibrations/second)											Mean frequency	Total mosquitoes used	Number of males not responding
		75	125	175	225	275	325	375	425	475	525	575			
15-17	Number of males responding	0	0	0	0	0	29	40	17	13	1		383.50	126	26
24-26		1	0	0	1	1	16	41	29	6	5		389.50	124	24
48-50		0	0	0	2	4	11	39	28	12	2	2	395.50	116	16
63-73		0	7	1	1	4	15	40	22	7	3		365.00	102	2
157-176		0	6	4	4	6	18	17	34	10	1		359.50	113	13
203-309		5	10	9	22	12	10	17	13	1	1		276.00	102	2
		Combined data													
15-50		1	0	0	3	5	56	120	74	31	8	2	389.50	366	66
		33%			1%	1.66%	18%	40%	24.66%	10.33%	2.66%	.66%			
63-176		0	13	5	5	10	33	57	56	17	4		362.25	215	15
			6.5%	2.5%	2.5%	5%	16.5%	28.5%	28%	8.5%	2%				

TABLE VII.—Lower Limits of Sound Frequencies which Induced the Mating Response in Six Different Age Groups of Non-virgin Male *aegypti*. (Males Kept with Females until Exposed to Sound; Age Group 15-50 Represents the Combined Data of Groups 15-17, 24-26, and 48-50; Age Group 157-309 is the Combined Data of Groups 157-176 and 203-309. The Percentages in Group 15-50 are Based on 150 Positive Responses, while those in Group 157-309 are Based on 100 Responses.)

Age of males (hours)		Sound frequencies (vibrations/seconds)											Mean frequency	Total mosquitoes used	Number of males not responding
		75	125	175	225	275	325	375	425	475	525	575			
15-17	Number of males responding	0	0	0	0	0	4	19	19	8			406	115	65
24-26		0	0	0	2	1	5	14	16	12			402	109	59
48-50		0	0	0	1	0	17	10	10	8	3	1	394	79	29
63-73		0	0	1	1	1	16	19	9	2	1		366	79	29
157-176		0	1	2	6	1	10	21	6	3			344	73	23
203-309		1	0	2	2	5	19	11	4	5	1		343	143	93
		Combined data													
15-50		0	0	0	3	1	26	43	45	28	3	1	400	303	153
					2%	.6%	17.3%	28.6%	30%	18.6%	2%	.6%			
157-309		1	1	4	8	6	29	32	10	8	1		343.5	216	116
		1%	1%	4%	8%	6%	29%	32%	10%	8%	1%				

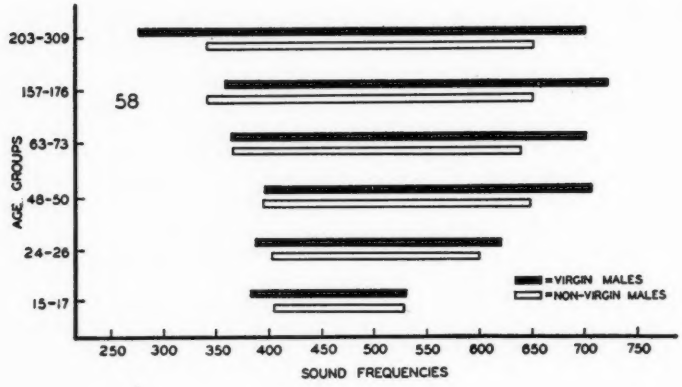
Discussion.—Figure 58 shows the range of sound frequencies, based on the mean upper and lower limits, which induced the mating response in 6 different age groups of virgin and non-virgin male *aegypti*. One point is apparent and common to both virgins and non-virgins. It is that with increased

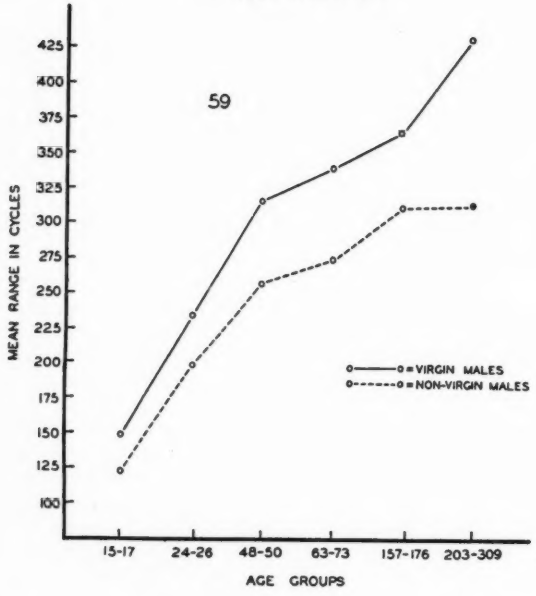


Figs. 56, 57.—56. Graph showing the lower limits of sound frequencies which induced the mating response in three age groups of virgin male *aegypti*. The age group 15-50 represents the combined data of the 15-17, 24-26, and 48-50 groups, and the 63-176 group represents the combined data of the 63-73 and 157-176 groups. (Data from Table VI); 57. Graph showing the lower limits of sound frequencies which induced the mating response in three age groups of non-virgin male *aegypti* (males kept with females until tested). The age group 15-50 represents the combined data of the 15-17, 24-26, and 48-50 groups; the 157-309 group represents the combined data of the 157-176 and 203-309 age groups. (Data from Table VII)

age there is a shift in both the upper and lower limits, so that the males respond to a greater overall range of sound. It is also apparent that the upper limits of the 4 oldest groups of non-virgins did not rise as high as the comparable age groups of virgins, nor did the non-virgin lower limit of the oldest group drop as low as the oldest virgin group. Thus the overall range of sound (i.e. difference between the mean upper and lower limits) which induces the mating response in non-virgins is narrower than that which stimulates virgin males. This is clearly shown in Fig. 59. The youngest virgin group responds to a mean range of only about 150 cycles and this range increases to about 425 cycles in the 203-309-hour-old males. The youngest non-virgin group responds to a mean range of about 125 cycles, with the oldest group responding to a mean range of about 310 cycles.

The sound limit determinations of different aged males show that a sound frequency which acts as a mating stimulus at one age does not necessarily stimulate the male at an earlier age. For example a 48-hour-old virgin male may respond to 775 cycles while one 26 or 17 hours old will not; or a 26-hour-old male will respond to 675 cycles while a 17-hour-old male will not. Adrian (1928, pp. 18, 19, 20) defined a stimulus as ". . . any change in the environment of an excitable tissue which, if sufficiently intense, will excite the tissue, i.e., will cause it to display its characteristic activity. The stimulus is thus the external change, and it may be ineffective—the stimulation may not have been intense enough to excite. . . . The stimulus must be a rapid change of conditions, and a gradual change will not do." Since the sound stimulus of 775 cycles which acted as a mating stimulus to a 48-hour-old or older male was the same as the sound which failed to stimulate a 17- or 26-hour-old male, the lack of response was either due to a difference in the condition of the receptor organ or to a change in the central nervous system. There is a difference in the external appearance of the antennae, with age, which can be correlated with the difference in the reception of different sound frequencies. It will be recalled that on emergence the antennal hairs of the male are flattened against the shafts (Figs. 9, 10) and apparently are incapable of vibrating to various frequencies. With increase in age these hairs become extended away from the shafts. Not all of these hairs are extended 24 hours after emergence, those on the apical half still remaining more or less recumbent and clinging together (Fig. 12). As more and more of these hairs become extended the male responds to higher and higher frequencies. After 48 hours practically all of the hairs are extended from the shafts (Fig. 13). It is perhaps significant that the 2 main shifts in the upper limits occur (1) before all of the antennal fibrillae are extended and (2) after the hairs become extended. Age groups older than 48 hours showed comparatively little fluctuation in their upper limits. Once the antennal fibrillae of *aegypti* become extended they remain more or less so throughout their lifetime. However, some old males were seen with some of the fibrillae on the apical half of the antennae partly recumbent and this may possibly account for some of the fluctuation in the upper limits of the 4 oldest groups tested. The correlation between the extension of the antennal fibrillae and the rise in the upper sound frequency limits indicates





Figs. 58, 59.—58. Graph showing the range of sound frequencies, based on mean upper and lower limits, which induced the mating response in six different age groups of virgin and non-virgin (males kept with females until tested) male *aegypti*. (Data from Tables IV-VII); 59. Graph showing the mean range of sound (difference between the mean upper and lower limits), expressed in numbers of cycles, which induced the mating response in six different age groups of virgin and non-virgin (males kept with females until tested) male *aegypti*. (Data from Tables IV-VII)

that the fibrillae on the apical half of the antennae covibrate to the higher frequencies, or a greater number of vibrating hairs are required to deflect the flagellum sufficiently for the higher frequencies to excite Johnston's organ. No conclusive experiments were performed to determine the limits of males lacking various numbers of antennal segments.

The shift in the lower sound frequency limits apparently cannot be correlated with the extension of the antennal fibrillae since the greatest drop takes place long after all of the antennal hairs become extended away from the shafts.

It is possible that the upper sound frequency limits of non-virgin males do not rise as high as virgins because of some form of conditioning by repeatedly copulating with females. It is not merely loss of virginity but continuous exposure to females which apparently affects the upper limits. It was noted that non-virgin males taken from very crowded cages containing both sexes, generally responded to lower frequencies in the determination of the upper limits (and often failed to respond at all), than non-virgin males taken from less crowded cages where presumably fewer copulations took place. Isolation of non-virgins from females also raised the upper limits of the males. Two examples can be noted. Twenty-five non-virgin males about 96 hours old and kept with females from emergence until exposed to sounds gave a mean upper limit of 601 cycles; these males were isolated from females and 48 hours later the mean upper limit for 20 of these individuals was 705 cycles. In another experiment, 25 non-virgin males, 47-48 hours old and kept with females from emergence until exposed to the sounds of the oscillator-speaker, gave a mean upper limit of 611 cycles; they were then isolated from females and their limits determined 38 hours later at which time the mean had risen to 693 cycles.

A parallelism may be drawn between the response of virgin males to a greater range of sound than non-virgins of the same age, with the feeding behavior of some polyphagous larvae. Brues (1946, p. 113) points out the fact that though gipsy moth larvae prefer oak foliage, "The lack of their preferred food and consequent hunger leads them to feed on less acceptable species, and finally as food grows scarcer they will show less and less choice, sometimes even turning to the needles of white pine which will serve the larger larvae, although the newly hatched brood are unable to utilize the pine foliage." The behavior of virgin male *aegypti* which have been isolated from females for some time may be the result of a form of "hunger" so that these "sex-starved" males respond to a greater range of sound than non-virgin individuals. The virgin males may be said to show "less choice" as to the sound wave with which they attempt to copulate.

Figure 60 compares the percentage of virgin and non-virgin males which failed to respond to sound in the determination of the upper and lower limits. A greater number of non-virgin males fail to respond to sound than do virgin males (non-virgin males which fail to respond to sound often copulate when placed in a cage containing females). For some reason, a higher percentage of

virgins and non-virgins failed to respond during the determinations of the lower limits than during the determinations of the upper limits. Only .5% of virgin males 48 hours and older failed to respond in the determination of the upper limits. These individuals are very responsive to the sounds used in determining the upper frequency limits which induce a mating response.

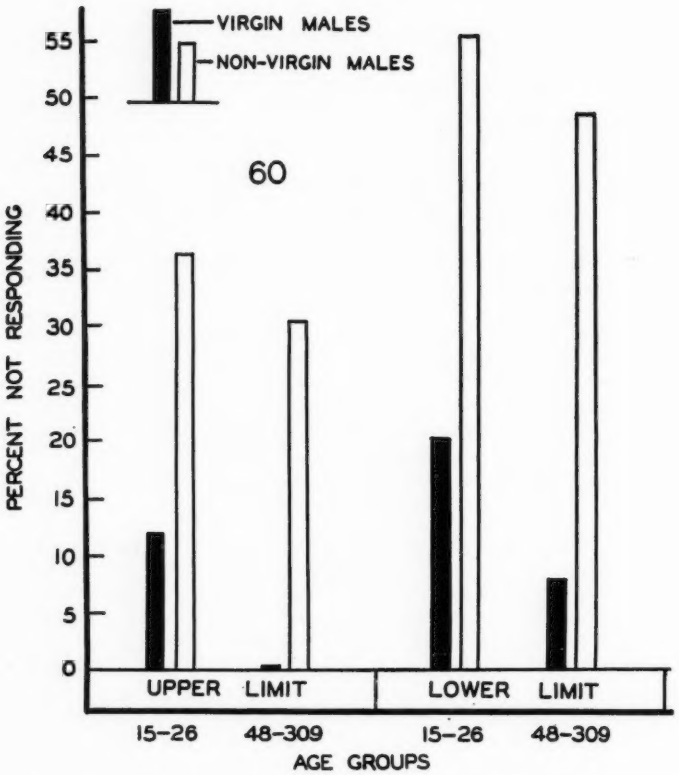


Fig. 60.—Graph showing the difference in the percentage of virgin and non-virgin male *aegypti* which failed to respond to sound in the determination of the upper and lower limits of sound frequencies which induced a mating response. The non-virgins were kept with females until the former were exposed to sound. The age group 15-26 represents the combined data of the 15-17 and 24-26-hour-old males; the 48-309 group is the combined data of the 48-50, 63-73, 157-176, and 203-309 groups. (Data from Tables IV-VII)

"ADAPTATION" OF MALES TO SOUND

Minnich (1925) found that he could "fatigue" *Vanessa* larvae to one sound frequency and then induce the insect to respond (i.e. throw the anterior end of the body dorsally or dorsolaterally) by changing to another frequency. He explained this as being due to different hairs reacting to specific sound vibrations. Male *aegypti* when repeatedly exposed to sounds of one frequency also cease giving a mating response but begin to respond again when the sound frequency is changed. It is possible that the cessation of response, after the male is exposed to a constant sound stimulus is not due to fatigue, but to adaptation. Hoagland (1936) defined sensory adaptation ". . . as a reversible reduction in the ability of a sensory cell, under the action of a constant stimulus, to initiate nerve impulses." Recovery of a nerve from fatigue is much slower than from adaptation and, in contrast to adaptation, is facilitated by the presence of oxygen. The present experiments do not determine whether the cessation of a mating response to a repeated stimulus is due to adaptation or fatigue. Adrian (1928, p. 68) stated that adaptation ". . . is a much more potent factor in causing the decline in frequency." The failure of male *aegypti* to give a mating response, after being subjected to continuous or intermittent sound stimuli, will be called adaptation.

When male *aegypti* were exposed to sounds from the audio oscillator-speaker, it was found that the insect could be adapted to one frequency and then made to respond again without a prolonged rest period, by changing the frequency of the sound stimulus. When subjected to continuous stimulation the male responds vigorously at first, but then the response becomes erratic and weak until he fails to respond at all. For example, a 12-day-old virgin male subjected to continuous stimulation of 600 cycles responded for about 2 minutes. After no response was given for one minute, the frequency was lowered to 400 cycles and the male began to respond again and continued to do so sporadically for 5 minutes. Although males can be adapted much more quickly by continuous than intermittent stimulation, their response is not uniform and it is difficult to decide when adaptation takes place. For this reason in the following experiments the males were subjected to sounds made for 3 seconds at 6-second intervals, and some tests were run with sounds made for 3 seconds at 2-second intervals and 10 seconds at 5-second intervals. The individual mosquitoes were exposed to the sounds in the same manner as those males which were used in determining the upper and lower limits of sound which would induce the mating response.

The clasping response to one sounding of a frequency may consist of one or more distinct flexings of the abdomen. Sometimes the response is a brief flexing of the abdomen followed by a return to the normal position, or the male keeps his abdomen flexed ventrad for several seconds. In the following tests one or more seizing responses, and one or more clasping responses regardless of the length of time the abdomen was kept in the flexed position, were

considered as one seizing and clasping response for each trial.[14] Only seizing or clasping responses given during the sounding of the particular frequency or during the brief silent intervals between the soundings were considered as positive for one trial. A male was considered adapted when he failed to respond to 10 consecutive soundings of a particular frequency. When this occurred the frequency was changed with no intervening rest period other than the fixed silent interval between soundings (i.e. 2, 5, or 6 seconds).

TABLE VIII.—Showing the Results of Subjecting *aegypti* Males to Sound Frequencies of 400 and 600 cycles, in a Long Series of Trials. C—Clasping Response; F—Male in Flight; N—No Response; S—Seizing Response; SC—Seizing and Clasping Responses. Virgin Males 12 Days Old. Each Frequency Sounded for 3 Seconds at 6-Second Intervals. Amplifier Control of Oscillator Reading 100.

Male No. 1			Male No. 2	
400 Cycles	(*Continued*)	(*Continued*)	*600 Cycles*	(*Continued*)
7 SC	2 SC	1 C	1 SC	3 C
1 C	1 C	1 SC	1 C	1 SC
1 SC	11 SC	1 C	3 SC	1 C
2 C	1 C	2 SC	34 C	1 SC
3 SC	4 SC	3 C	1 SC	1 N
6 C	1 C	4 SC	2 N	2 C
1 SC	2 SC	1 C	1 S	1 N
1 C	1 C	5 SC	1 N	1 C
1 F	2 F	3 S	3 C	1 N
1 SC	1 S	1 C	1 S	1 S
1 F	7 SC	3 SC	3 SC	3 C
3 SC	1 C	1 F	1 C	1 S
1 C	8 SC	5 C	1 N	1 N
1 SC	1 C	1 SC	4 C	1 C
1 C	8 SC	1 C	1 N	1 SC
2 SC	1 C	2 SC	8 C	3 C
1 C	1 N	1 N	1 SC	1 SC
1 SC	1 C	1 C	11 C	3 C
2 C	1 SC	3 SC	2 N	1 S
1 F	3 C	1 C	1 S	3 C
3 C	3 SC	2 SC	1 C	1 SC
2 SC	1 C	7 C	3 N	2 C
1 C	3 SC	1 F	1 SC	(discontinued; not
2 SC	2 C	1 SC	1 C	adapted after
1 C	8 SC	1 C	1 N	122 trials)
4 SC	1 C	2 SC		
1 C	5 SC	1 C		
3 SC	1 C	1 SC		
1 C	5 SC	1 C		
1 SC	2 C	(discontinued; not		
1 C	2 SC	adapted after		
		207 trials)		

14 A single sounding of a frequency followed by its silent interval represents one trial.

Males vary in the length of time required to adapt to a particular frequency. Table VIII shows the results of exposing 2, 12-day-old virgin males to 400 and 600 cycles respectively, over a long series of trials. Male number 1 was not adapted to 400 cycles after 207 trials at which time the tests were discontinued. In this case the first negative response was obtained after 110 positive responses. Male number 2 exposed to 600 cycles was not adapted after 122 trials at which time the experiment was discontinued. In this case the first negative response occurred after 40 positive responses.

Table IX shows the results of adapting males to one frequency and then changing to another frequency. Male number 3 became adapted to 400 cycles after 39 trials, to 500 cycles after 54 trials, and to 600 cycles after 68 trials. It can be seen that this male gave a high number of negative responses (44 N's) before becoming adapted to 600 cycles per second. Male number 4, after becoming adapted to 500 cycles, failed to respond to 5 soundings of 600 cycles before giving the first response to that frequency. Tables VIII and IX show the results of only 4 of the dozen males tested.

Pumphrey (1936) was able to adapt the afferent nerve of tactile hairs of the roach by deflecting the receptor with a fine glass needle. It is possible that in *aegypti* different sound frequencies vibrate different numbers of antennal fibrillae which in turn cause the antennal shaft to deflect or vibrate at its base to a greater or lesser extent. Certain sense cells of Johnston's organ may be stimulated by the deflection of the antenna when exposed to sounds of 400 cycles and after these cells become adapted the male ceases to respond. When the frequency is changed to 500 cycles, differential vibrations of the shafts results in other sense cells being stimulated and the male again responds until these cells become adapted. Some tests performed on young males about 25 hours old (sounding each frequency for ten seconds at five-second intervals) indicated that when adapted to 400 cycles they will not respond to 500 and 600 vibrations per second. This may possibly be correlated with the condition of the antennal hairs inasmuch as not all of the fibrillae are extended away from the shafts at this time.

The Behavior of Males of Other Culicidae to Sound

Males of *A. quardimaculatus* which are in a mating-state (determined by the extension of the antennal fibrillae and their attempts to copulate with females) may show a mating response to tuning forks with the following frequencies: 320, 341.3, 384, 426.7, and 480 vps. The response is somewhat similar to that given by *aegypti* males though usually it is not so vigorous. *Quadrimaculatus* males give either the seizing or clasping response, or both. One interesting case may be noted. A male was induced to give the clasping response and in so doing his genitalia succeeded in clasping the cloth of the cage; he then released his hold with his front legs and leaned backwards propped up by his hind legs. This response is comparable to the release of the leg hold on the female once connection is made. It has previously been suggested that the end-to-end position is an adaptation resulting from the reduc-

TABLE IX.—Results of Adapting *aegypti* Males to the Sounds of Three Different Frequencies over a Long Series of Trials. C—Clasping Response; F—Male in Flight; N—No Response; S—Seizing Response; SC—Seizing and Clasping Responses. Virgin Males 12 Days Old. Each Frequency Sounded for 3 Seconds at 6-Second Intervals. Amplifier Control of Oscillator Reading 100.

Male No. 3		Male No. 4	
400 cycles	(continued) *600 cycles*	*400 cycles*	(continued)
2 SC		3 SC	1 SC
4 S	1 C	3 C	2 N
1 SC	1 S	7 N	1 S
2 C	3 N	2 S	1 C
1 SC	1 C	2 SC	1 SC
7 C	2 N	1 C	2 C
1 SC	1 F	4 SC	3 N
3 C	1 S	1 N	1 C
1 N	1 F	1 C	1 N
10 C	2 N	1 N	2 C
2 N	2 S	1 SC	1 N
3 C	1 N	3 N	1 C
1 N	2 C	2 C	2 N
1 C	1 SC	1 SC	15 SC
10 N	1 C	1 C	2 C
(adapted after 39 trials)	1 N	3 N	8 SC
	1 S	1 C	10 N
	9 N	1 S	(adapted after 78 trials)
	2 C	1 SC	
500 cycles	6 N	1 N	
	1 C	3 C	
1 C	7 N	1 SC	*600 cycles*
1 SC	2 C	3 C	
7 C	1 N	1 N	5 N
1 N	2 C	2 C	11 C
3 C	7 N	10 N	1 SC
1 N	1 C	(adapted after 50 trials)	4 C
2 C	3 N		(discontinued; not adapted after 21 trials)
1 SC	1 C		
1 C	2 N		
1 S	2 C	*500 cycles*	
1 N	10 N		
3 C	(adapted after 68 trials)	1 SC	
1 N		8 C	
3 C		1 SC	
1 SC		1 C	
1 N		1 SC	
10 C		6 C	
1 N		1 S	
4 C		1 SC	
1 N		2 C	
7 C		1 SC	
1 S		9 C	
1 SC		1 S	
10 N		1 C	
(adapted after 54 trials)			

tion of the tarsal claws of the male making it more difficult for him to cling to the female, since only the front tarsi are suited for seizing and holding. It would appear from the above observation that clasping the female genitalia may be the stimulus which induces the male to release his front leg hold.

Nuttall and Shipley (1902, p. 77) obtained negative results after attempting to study the effects of electromagnetically excited tuning forks upon male *A. maculipennis* and they believed this was possibly due to the males having been confined for some days in small boxes together with females. The present positive responses to tuning forks were obtained from *quadrimaculatus* males which were confined with females in the same cage and were attempting to copulate with them or from males isolated from females but which had their antennal fibrillae extended and were active. It is likely that Nuttall and Shipley's negative results were due to the fact that the males exposed to sound vibrations were not in a mating-state. These writers did not indicate the condition of the male antennae during their tests, again apparently not realizing the significance of the extension of the antennal hairs. It is also possible that the sounds they used were not frequencies which would stimulate the species with which they experimented. Bates (1941) observed that *A. superpictus* swarms did not respond to tuning forks, sounded in a room-sized cage. Here it is possible that the frequencies used were not within a range which would stimulate this species, or the forks may not have been struck close enough to the swarms to be effective.

Four- and 6-day-old reared males of *Psorophora confinnis* (Lynch-Arr.) were exposed to the sounds of the audio oscillator-speaker. The antennal hairs of these individuals were more or less recumbent and clung together against the shafts. In general, the males tested were very unresponsive to sounds, responding only to frequencies between 260 and 310 vibrations per second. It is probable that the range of sound to which males of this species respond is greater in those males the antennal fibrillae of which are all extended. In comparison, the mating response of *confinnis* males is less vigorous than that shown by *aegypti*. Only a clasping response was given. The rapid vibration of the wings and body-shaking characteristic of the *aegypti* response was not observed.

Culex pipiens males which were attempting to mate with females (i.e. were in a mating-state), gave a seizing or clasping response, or both, when exposed to the sounds of the tuning forks 288, 320, 341.3, and 384 vps.

The Practical Application of the Behavior of Mosquitoes to Sound

Using fly-paper and also devising an electrocuting apparatus, Weaver (Howard, 1901) was able to attract and capture or kill the mosquitoes in the vicinity by sounding a particular note. The sound frequency, species, and sex of the mosquitoes attracted were not indicated. Laurence (1902) recalled that as a child he and his friends would attract mosquitoes (species and sex not indicated) by the sound of the voice and kill them (presumably by hand).

Nuttall and Shipley (1902) believed that the knowledge gained from a study of the effect of sound on various species of Culicidae ". . . might be ultimately put to practical use." Kahn et al. (1945) recorded the various sounds produced by several species of culicids with the intention of reproducing these sounds to attract and kill the mosquitoes. A recent popular magazine[15] indicated that Dr. Kahn was ". . . preparing to leave for Africa, where he will try to trap and destroy disease-bearing mosquitoes by playing back to them some recordings of their own love calls."

There is no question that *aegypti* males are attracted by certain sound frequencies and will continue to be attracted throughout their lifetime once they are capable of perceiving sound. However, males of *quadrimaculatus* and *pipiens*, and probably those of other species which swarm, are indifferent to sounds unless they are in a mating-state and this occurs only periodically. Even males of these last two species which are attempting to copulate with females (in the laboratory) appear to be less responsive than *aegypti* to the sounds of tuning forks in the sense that a mass response (attraction) to a stimulating sound freqeuncy was not observed. Actually it would be unnecessary to play back "love calls," as suggested by Kahn et al., to attract males since they (i.e. *aegypti*) respond to a rather wide range of sound frequencies.

Though the literature suggests that females of some species of mosquitoes are attracted by and enter male swarms, no evidence was found in the present experiments to indicate that females of *aegypti* are attracted by the sound of the males. There is no experimental proof in the literature that females are attracted by the sounds of male culicids. If sound is used as a means of attracting and killing mosquitoes, all indications are that males would be the sex destroyed. It should be remembered that our mosquito-borne diseases are transmitted by females of the species. It is conceivable that destroying a large percentage of males would decrease the number of impregnated females(provided the males were destroyed before mating) and over a period of time would result in the population being reduced to a point where the species would be medically unimportant. However, until further study, it appears unlikely that a killing device employing sound as an attractant would be a more efficient or economical substitute than the present methods of mosquito control.

Summary and Conclusions

Unless otherwise stated, the following statements concern *Aedes aegypti*.

1. Copulation is initiated during flight. The male usually seizes the female from above, reorients himself, and assumes the modified linear pose. Copulation is concluded in the modified linear position. The act may be completed with the pair in flight or usually after the female comes to rest with the male still clinging to her. The pair remains joined in coitu briefly, connection lasting about 4 to 59 seconds.

15 "Hot Platters by Mosquitoes," an anonymous note in Science Illustrated, 2, No. 5 (May, 1947), p. 55.

2. Males will copulate repeatedly with the same or different females. One male may fecundate more than one female but the fact that a male has copulated with a female does not imply that she has been impregnated. A female which has copulated once or twice usually has only two of her three spermathecae filled with sperm.

3. The sound produced by the female is the external stimulus which attracts the male and induces him to copulate. Odor and sounds other than those made by the female in flight apparently play no rôle in mating behavior. Any factor which causes both sexes to fly (e.g. increased temperature, agitating the cage, etc.) will automatically increase the frequency of copulations.

4. The male responds to a range of sound frequencies rather than to a single frequency. Male *aegypti* attempt to copulate with males and females of several different species of Culicidae because the sounds made by these mosquitoes fall within a stimulating range. Sexually active males cannot differentiate between recently emerged males and old females since the sounds made by very young males in flight fall within the spectrum which will induce copulation.

5. The male palps and proboscis take no part in copulation.

6. The male antennae are the organs used to locate the female in flight. At emergence the male antennal fibrillae are recumbent and cling together against the shafts. The fibrillae become extended as the insect ages. Usually all of the fibrillae are extended about 48 hours after emergence. Once the antennal fibrillae of male *aegypti* become extended they remain more or less so throughout the lifetime of the insect.

7. The male genitalia must undergo about a 180 degree rotation before copulation can be successful. However, males may attempt to copulate when their genitalia have rotated only about 135 degrees though these attempts generally are unsuccessful because of mechanical difficulties. Males may give a typical mating response to the sounds of a tuning fork as early as 5 hours 20 minutes after emergence, long before they are physically capable of copulating due to incomplete rotation of the genitalia. Male mating behavior elicited by a female in flight is not correlated with rotation of the genitalia.

8. The induction of male mating behavior apparently is dependent upon the condition of the antennae. The male attempts to copulate when a sufficient number of its antennal fibrillae are extended making possible the perception of the sound of the flying female. This occurs about 15 to 24 hours after emergence and by this time his hypopygium has rotated sufficiently so that copulation can be accomplished successfully. Since mating is initiated in flight and the stimulus is the sound of the flying female, copulation will occur only when the female begins to fly voluntarily at least several hours after emergence. By this time she becomes "attractive" because the sound made in flight falls within the range which will stimulate the male. Very young females can be made to fly unmolested in a cage containing sexually active males since the

sound produced by a recently emerged female in flight falls outside of the range which will induce the male to copulate. Whether the stimulus which first induces the female to fly is sexual or nutritional (i.e. the search for food) was not determined. No evidence was found to indicate that the female is attracted to the male. Investigators have shown that the average emergence period of *aegypti* females is about 14 hours longer than that of the males. Thus by the time the female begins to fly the males are ready to copulate by reason of their "matured" antennae and rotated genitalia.

9. Once male *aegypti* begin to copulate (i.e. 15 to 24 hours after emergence), they continue to do so at any time of the day, until death, whenever stimulated by flying females. These males may be said to be in a constant mating-state throughout their lives. The males of *Anopheles quadrimaculatus* mate cyclically and between mating periods ignore females. During inactive periods the antennal fibrillae of male *quadrimaculatus* lie recumbent against the shafts. When these males are sexually active, their antennal fibrillae are extended away from the shafts. The males of *Culex pipiens* also mate periodically; however, unlike *quadrimaculatus,* the antennal fibrillae of sexually inactive male *pipiens* remain extended away from the shafts.

10. The front and middle tarsi of the male are structurally suited for seizing and holding the female. The middle tarsi and claws appear to function chiefly in the initial seizure. The front legs are used in both seizing the female and in gaining the modified linear pose required for copulation. The male hind tarsi are not suited for seizing and take comparatively little part in mating.

11. Except for flight, the female is passive while being seized by the male, and her legs remaining outstretched serve as structures to which the male can cling. The female tarsi are not suited for seizing in spite of the toothed condition of the claws, and she does not cling to the male or hold him to her during copulation. Normal male *aegypti* copulate readily with females which have all their last tarsal segments removed.

12. The hypothesis is suggested that the face-to-face and end-to-end positions assumed by different species of mosquitoes might be correlated with the degree of development of the last tarsal segments and claws of the male's front and middle legs. *A. quadrimaculatus* males with only one pair of legs suited for seizing, initiate copulation in the modified linear pose, release the female almost immediately after making connection, and swing out into the linear position. The attitudes of *C. pipiens* appear to represent a transitional state where the pair may remain joined for awhile in the face-to-face position and finally concludes copulation in the opposed position. In this species both the front and middle male tarsi are fairly well fitted for seizing. The front and middle legs of male *aegypti* are well suited for seizing and this species was never seen to copulate end-to-end (sometimes an opposed position is assumed while the pair tries to separate after completing copulation). The front tarsi of *aegypti* appear to be fitted better for seizing than the same legs of *pipiens.*

13. It is suggested that the mating behavior of *aegypti* may have evolved from a species which originally swarmed to that shown today, where the males generally do not form swarms and may copulate even without a preliminary "dance."

14. The instinctive mating behavior of *aegypti* males whether elicited by a female mosquito or by mechanically produced sounds is basically similar. These coordinated reflex actions, depending upon the source of the stimulus, may be tabulated as follows:

Source of stimulus	*Behavior of male*
A. Female in flight	1. Flight (if male is at rest) 2. Attraction 3. Seizure of female 4. Clasping the female genitalia
B. Tuning fork (of proper frequency)	1. Flight (if male is at rest) 2. Attraction 3. Seizing response 4. Clasping response

The seizing response elicited by the sounds of a tuning fork or audio oscillator-speaker consists of clinging with the front and middle legs to the cloth of the cage, near the sound source, and vibrating the wings rapidly. The clasping response which usually follows the seizing response consists of flexing the abdomen ventrad so that the genitalia touches the side of the cage.

15. Males also respond to certain sound frequencies by giving so-called "shock-reactions."

16. Experimental evidence is presented to confirm the hypotheses that in Culicidae Johnston's organ is aural in function and that the mode of action of this structure is that described in the literature. These hypotheses were previously based solely on morphological evidence.

17. The limits of sound frequencies which will induce a mating response from *aegypti* males are about 100 and 800 cycles per second. The limits vary with the age and virginity or non-virginity of the males. Both virgin and non-virgin males respond to higher frequencies, with age, the shifts occurring largely between 17 and 24 hours, and 24 and 48 hours after emergence. These shifts in the upper limit can be correlated with the extension of the antennal fibrillae. With the extension of more and more fibrillae the male is able to respond to higher frequencies. The lower limit of sound frequencies which will induce a mating response in virgins drops markedly after males are isolated from females for 203-309 hours. With increase in age, both virgin and non-virgin males respond to a wider range of sound, but the range of non-virgins, which have copulated repeatedly, is narrower than that of virgin males kept isolated from females. Males can be "adapted" to one sound frequency so that after continuous stimulation they fail to give a mating response. They can be made to respond again by changing the frequency of the sound stimulus. Pos-

sible explanations for the phenomenon of "adaptation" and the response to a wider range of sound with increase in age are discussed.

18. *A. quadrimaculatus* and *C. pipiens* when in a mating-state, give a mating response to the sounds of tuning forks. *Quadrimaculatus* males respond to the 320, 341.3, 384, and 426.7 forks. *Pipiens* males give a mating response to sounds from the 288, 320, 341.3, and 384 tuning forks. *Psorophora confinnis* males give a mating response to oscillator-speaker sounds between 260 and 310 cycles per second (these males did not have all their antennal fibrillae fully extended). The limits of sound frequencies which will induce the mating response in the above three species of mosquitoes were not determined.

19. No evidence was found that female *aegypti* are attracted to sounds though they may give shock-reactions to certain frequencies.

20. With respect to mating, the functions of the male antennae are (1) to perceive the sound of the flying female, (2) to locate the direction of the sound source, and (3) to discriminate between certain sound frequencies since some sounds may induce shock-reactions while others act as a mating stimulus.

21. At present it seems unlikely that killing adult mosquitoes with the aid of a sound-attractant device would be more efficient and economical than the current methods of mosquito control.

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The Food of the Bridled Shiner, Notropis bifrenatus (Cope)[1]

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In the Oyster River at Durham, New Hampshire, the chief predator of the bridled shiner, *Notropis bifrenatus* (Cope) is the pickerel, *Esox niger*. Throughout the season when bridled shiners were in evidence, they were the most abundant fish in the shallows where pickerel up to a foot and one half in length were very common. Bailey (1938: 169) regarded the bridled shiner, where it is found in New Hampshire, as a major item of the pickerel diet. Gordon (1937: 110) considered it to be a fine forage fish for the small mouth bass, *Micropterus d. dolomieu*, in the Saco watershed of New Hampshire. According to Webster (1941: 160-161) "the extent to which the bridled minnow serves as forage is somewhat indefinite. Its abundance in Connecticut falls considerably below that of the golden shiner, but since it inhabits relatively shallow water throughout life, this minnow may be an important food for young bass and pickerel." Greeley and Greene (1931: 87) state that "this minnow is the most abundant small fish available as food for the young northern pike which live in the stream (Tibbit's Creek near Ogdensburg, N. Y.) during their first summer. The small size, abundance, and habit of living in marsh areas make this minnow a good food fish for the young of the northern pike, muskalonge, and probably other game fish." Greeley (1940: 74) found it associated frequently with the northern pike and large mouth bass in the Lake Ontario watershed of New York. Abbott (1878: 834) found the striped bass, *Roccus lineatus*, feeding on bridled shiners in the Delaware River.

Manner of Feeding

The bridled shiner was under observation on eighty-nine different days at the Oyster River in Durham, N. H., from the latter part of March to the middle of August, 1946. It forages in currents just sufficient to compel it to face upstream and exert itself a little to maintain its position, and from which it departs from time to time to dart after morsels of food. However, it prefers still water, where it feeds from plant surfaces, on the bottom, and on plankton in shallow open waters over beds of submerged vegetation. Bottom feeding prevails when and where the vegetation is sparse or lacking, and especially during early spring, although it went on to a considerable extent throughout the period of observation. When feeding on the bottom, it lunges at specific prey, turning over on its side and stirring up a slight cloudiness as it seizes the organism, or it nibbles at objects on the bottom, making an angle of 45°

1 Abstracted from a doctoral dissertation prepared under the direction of Professors A. H. Wright and E. C. Raney of Cornell University, Ithaca, N. Y.

with the bottom as it moves along. Chub sucker, *Erimyzon o. oblongus* yearlings of the same size appear to feed with less discrimination, but take in and reject much bottom material, and they do not feed as uninterruptedly as bridled shiners do. With the growth of plants, the bridled shiner forages mostly among the weeds, often down in depressions in the plant mass, where it browses on epiphytes and other organisms closely associated with the plants. By midsummer, larger individuals tend somewhat to plankton feeding, and are seen swimming up and down casually, sampling, ingesting, or rejecting minute particles of food suspended in the water, pausing to nibble at plant surfaces a greater part of the time, and occasionally descending to feed on the bottom. In waters up to two and one-half feet deep, golden shiners, *Notemigonus c. crysoleucas* of the same size feed mostly on plankton, from mid-level to surface, bridled shiners, from mid-level to bottom, and chub sucker young, on the bottom and sometimes from plant surfaces at higher levels. Feeding goes on during all the daylight hours, but was never observed after dark. Both sexes forage even over the spawning beds during the intervals when breeding activity is temporarily slack. Out of 219 stomachs examined, only 19 were empty. Often bridled shiners are seen with young chub suckers foraging in the nests of common sunfishes. The sunfish repeatedly drive them off.

MATERIAL AND METHODS

In order to afford a representative sampling of the food of this species, specimens were taken throughout the season as indicated in Table I. Specimens hatched and reared in an aquarium attained a standard length of 9 mm. in approximately three weeks. Specimens as large as 45 mm. in standard length are rare. The greatest size ever recorded for this species was 48 mm. in standard length. The Ithaca collections were made with a 10 or 20 foot seine; the Durham collections with a 4 foot seine supplemented with a fine-meshed hand net. The sex of each specimen was determined when possible, but there was no apparent correlation between sex and food selection. The standard length was measured to the posterior end of the hypural. Each stomach was removed from the fish and placed in a watch glass, the contents sorted, identified, and the volume of each category of food estimated. The contents of the intestine were not included. An analysis of the stomach contents of the 200 specimens is presented in Table II.

SUMMARY OF THE FOOD

Animals were found in 198 (99%) of the stomachs, and accounted for 73.4% by volume of all items, whereas plant materials were found in only 50 (25%) of them, and constituted but 6% of the volume of all items. Plant material was mostly filamentous algae with some fragments of vascular plants, and in one collection, *Chara* oögonia. Insects and entomostracans together made up the bulk of the animal food. They occurred with about the same frequency (73.5% and 74.0% respectively), and together constituted 60.4% of the volume of all items, leaving only 10.9% for all other animal food

(exclusive of parasitic helminths). Of this 10.6%, amphipods and hydrachnids accounted for 7.1% and 3.3% respectively, the remainder, 0.5%, being comprised of unidentified arthropod fragments (found in 2 stomachs), molluscs (in 3), and Protozoa (in 6). Although insects and entomostracans occurred with about the same freqeuncy, insects contributed 36.4% of the volume of all items, whereas entomostracans contributed 24%.

TABLE I.—Collection data for specimens of *Notropis bifrenatus* (Cope) used for stomach analysis.

Date	No. of specimens	Range in standard length	Locality
Mar. 21, 1938	20	28-45 mm.	Stewart Pk. lagoon, Ithaca, N. Y.
Apr. 10, 1946	17	19-32 mm.	Longmarsh Brook, Durham, N. H.
Apr. 22, 1946	21	18-31 mm.	Oyster River, Durham, N. H.
May 17, 1941	20	26-30 mm.	Fall Creek, Ithaca, N. Y.
June 21, 1941	20	21-31 mm.	Cayuga Lake, Ithaca, N. Y.
June 26, 1942	11	23-33 mm.	Oyster River, Durham, N. H.
July 7, 1942	20	24-35 mm.	Oyster River, Durham, N. H.
July 22, 1942	19	9-18 mm.	Oyster River, Durham, N. H.
Aug. 18, 1942	20	14-36 mm.	Oyster River, Durham, N. H.
Oct. 24, 1936	32	22-44 mm.	Stewart Pk. lagoon, Ithaca, N. Y.
Total	200	9-45 mm.	

INSECT FOOD

The order of importance of insect food, measured by percentage of the volume of all items, was as follows: Chironomidae, 17.1%; Psychodidae, 7.9%; Trichoptera, 4.2%; Hemiptera, 1.7%; Simuliidae, 1.4%; Ceratopogonidae, 1.0%; Coleoptera, 0.9%; Plecoptera, 0.5%; Ephemeroptera, 0.1%. The family Chironomidae is rivalled in importance among all the food organisms only by the Cladocera. The chironomids were all larvae. No attempt was made to determine genera or species, although the genus *Pentaneura* of the Tanypodineae was recognized. The Psychodidae were represented by pupae, many apparently of the genus *Pericoma*. Among the Ceratopogonidae, *Bezzia setulosa* larvae were present. The family Simuliidae was represented by the larvae of one species, *Prosimulium hirtipes* found only in specimens from Longmarsh Brook, taken April 10. Plecoptera and Ephemeroptera nymphs were found only in one stomach, of one of the largest specimens of the March collection. Trichoptera, the only insect order other than Diptera present in significant amounts, was represented mostly by the family Hydroptilidae. Some Psychomiidae had been taken, among which *Polycentropus interruptus* and *Phylocentropus* sp. were identified. The Coleoptera were represented by *Hydroporus* sp. of the Dytiscidae, and by a few unidentified fragments. Hemiptera contributed mostly a few tiny unidentified nymphs except for the August collection, in which the family Corixidae comprised 17% of the volume of all items, and occurred with a frequency of 40%.

TABLE II.—Summary of the food of 200 bridled shiners, *Notropis bifrenatus*, ranging from 9 to 45 mm. in standard length, selected from collections made in March, April, May, June, July, August, and October.[1]

Item	Stomachs in which the item was found		Percentage (estimated) of the volume of all items
	Number	Percent	
DEBRIS	93	46.5	20.6
PLANT	50	25.0	6.0
Algae	43	21.5	5.1
Other	11	5.5	0.9
ANIMAL	198	99.0	73.4
Insecta	147	73.5	36.4
Plecoptera	1	0.5	0.5
Ephemeroptera	1	0.5	0.1
Hemiptera	11	5.5	1.7
Trichoptera	18	9.0	4.2
Coleoptera	5	2.5	0.9
Diptera	130	65.0	28.8
Psychodidae	43	21.5	7.9
Simuliidae	7	3.5	1.4
Chironomidae	112	56.0	17.1
Ceratopogonidae	10	5.0	1.0
Unidentified Diptera	10	5.0	1.4
Unidentified Insecta	1	0.5	0.2
Hydrachnida	30	15.0	3.3
Amphipoda	33	16.5	7.1
Entomostraca	148	74.0	24.0
Copepoda	90	45.0	7.1
Cladocera	104	52.0	15.1
Ostracoda	19	9.5	1.8
Unidentified Arthropoda	2	1.0	0.2
Mollusca	3	1.5	0.2
Acanthocephala	4	2.0	0.9
Nematoda	10	5.0	0.3
Trematoda	16	8.0	0.9
Protozoa	6	3.0	0.1

1 See Table I.

ENTOMOSTRACAN FOOD

Of the 24% of the volume of all items composed of entomostracans, 15.1% was Cladocera (only 2% less in amount than the most prevalent item of animal food, Chironomidae), 7.1% was Copepoda (equal in amount to the Amphipoda), and 1.8% was Ostracoda. Most of the Copepoda were *Cyclops* sp.; a few were of the genus *Canthocamptus*. The frequency of occurrence of the Ostracoda was only 9.5% in contrast to 45% for the Copepoda and 52%

for the Cladocera. Among the Entomostraca, Cladocera were best represented, not only by frequency of occurrence and by percentage of the volume, but also by diversity of species. The Cladocera identified belonged to three families: Chydoridae, Daphnidae, and Bosminidae. Chydoridae were present in 83 stomachs, Daphnidae in 11, and Bosminidae in only 2. Unidentified Cladocera were found in 12. *Bosmina longirostris* was the only member of the Bosminidae present. Of the Daphnidae, examples of *Ceriodaphnia* were encountered in collections of three dates, those in one being *Ceriodaphnia reticulata*. *Moina* and *Daphnia* each occurred in stomachs from one collection. The Chydoridae made up the greater part of the total volume of all Cladocera. *Eurycercus lamellatus*, one of the largest and least fragile of the Cladocera, was present in stomachs from 8 out of the 10 collections. *Acroperus harpae* was found in specimens from 4 collections, *Chydorus sphaericus* in those of 3, and *Camptocercus rectirostris* in those of 2. Each of the following were found in fish from one collection: *Leydigia* sp., *Alona affinis*, *Graptoleberis testudinaria*, and *Simocephalus serrulatus*.

FOOD OF THE YOUNG

In Table III is presented an analysis of the food of 19 *Notropis bifrenatus* young of the year, ranging from 9 to 18 mm. in standard length, and collected on July 22, 1942 in the Oyster River. Comparing this with the analysis of the food of the 200 specimens as a whole (Table II), it will be seen that the volumetric ratio between plant and animal food is much the same, and substantially the same categories of food are present. Although only three orders of insects are represented, Diptera with the family Chironomidae again lead in importance among the insects, with the order Trichoptera in second place. Also, among the entomostracans, Cladocera, Copepoda, and Ostracoda occupy the same relative positions. However, in the stomachs of the young, insects compose only 14.8% of all the animal food, whereas in the 200 specimens as a whole, they compose 49.5%. In the young, entomostracans make up 80.5% of the volume of all animal food, whereas for the 200 specimens as a whole they make up only 32.7% of all the animal food.

SEASONAL VARIATIONS IN THE FOOD

With the exception of the collection of young just referred to, the analyses of the stomach contents for each collection composing the 200 specimens are arranged in Table IV in the order of the time of year the collections were made. To permit an easier comparison of the seasonal variations in food selection, these data are plotted in the graph in Fig. 1. The effect of habitat or location is apparent in that all the specimens from Ithaca, N. Y. contained considerable debris. The debris was partly silt and partly flocculent organic material, and occurred in the alimentary tracts of the food organisms as well. There appears to be a positive correlation between the frequencies with which debris, plant material and mites (Hydrachnida) were taken. The relative abundance and frequency of occurrence of amphipods in the stomachs of the bridled shiner show no obvious correlation with fluctuations in the occurrence

of other food items. Uniformly scarce in stomachs from Ithaca, in Durham specimens, they show an increase in late April and a peak in June with a decline in July and August. This may have been due to fluctuations in the abundance of amphipods or to the fact that bridled shiners foraged most commonly among vegetation during the period when amphipods were well represented in their food.

Insects and entomostracans made up the bulk of the food on all but a few dates, with insects usually constituting the greater part of the volume. Debris made up a considerable proportion of the bulk on many occasions, and probably contained some small perishable oragnisms. On a few dates, amphipods exceeded all other items in volume, but mites and plant material although present in significant amounts at different times never contributed a sizable proportion of the food. The remaining categories of organisms amounted to only 2.6% of the volume of all items, and of this, 2.1% consisted of helminth parasites rather than food.

No previous intensive studies of the food of this species have been published. Rimsky-Korsakoff (1930: 91) examined thirty specimens from the Lake Champlain watershed of New York. Eight specimens, 32-35 mm. long, contained only Cladocera. Fourteen, 32-48 mm. long, contained 30% Clado-

TABLE III.—Summary of the food of 19 *Notropis bifrenatus* young of the year, ranging from 9 to 18 mm. in standard length, and collected July 22, 1942 in the Oyster River, Durham, N. H.

Item	Stomachs in which the item was found		Percentage of the (estimated) volume of all items
	Number	Percent	
PLANT	4	21.1	9.7
Algae	4	21.1	9.7
ANIMAL	19	100.0	90.3
Insecta	6	31.6	13.5
Hemiptera	1	5.3	0.1
Trichoptera	1	5.3	1.8
Diptera	5	26.3	11.6
Psychodidae	1	5.3	1.7
Chironomidae	5	26.3	9.9
Hydrachnida	1	5.3	1.1
Entomostraca	19	100.0	72.7
Copepoda	16	84.2	18.3
Cladocera	16	84.2	53.7
Ostracoda	2	10.5	0.7
Nematoda	2	10.5	1.1
Trematoda	4	21.1	1.9

cera, 25% Copepoda, and 45% Ostracoda. Among the crustaceans present were: *Chydorus sphaericus, Sida crystallina, Alona* sp., and *Cyclops bicuspidatus*. Eight specimens, 35-39 mm. long, contained 40% Cladocera, 10% Copepoda, and 50% miscellaneous, including chironomid larvae, and Hydracarina. Among the crustaceans present were: *Ceriodaphnia* sp., and *Sida crystallina*. Carpenter (1930: 197, 203), out of nineteen stomachs from specimens collected in the Bouquet River of the same watershed, found one with chironomid larvae only, five with 90% land insects and 10% fiber, two with dipterous larvae, six with Entomostraca, and five with chironomid larvae and *Ephemerella* nymphs. Sibley (1931: 117) examined thirty stomachs from specimens taken in the St. Lawrence watershed of New York. Two, 33 mm. long, taken from Branch Brook on June 5 contained: 5% animal and 35% plant plankton, 20% aquatic insects, and 40% molluscs; fifteen specimens, 35 mm. long, taken from Line Brook, June 7: 25% animal and 35% plant plankton, and 70% aquatic insects; thirteen specimens, 30-39 mm. long, from

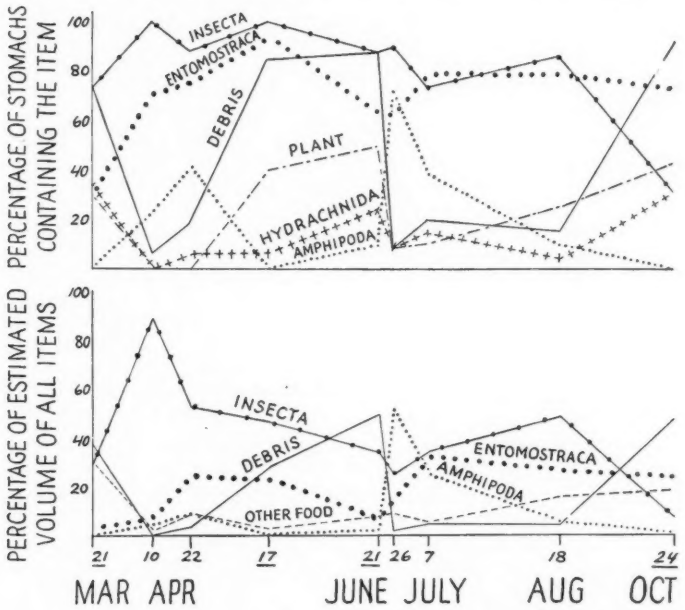

Fig. 1.—Seasonal changes in the food of the bridled shiner, *Notropis bifrenatus* (Cope) plotted from data in Table IV. The dates of collections made at Ithaca, N. Y., are underlined. In the upper graph, the percentages of stomachs containing the respective items are plotted. In the lower graph, the percentages (estimated) of the total volume contributed by the respective items are plotted. "Other food" includes: plant material, hydrachnids, unidentified arthropods, molluscs, helminth parasites, and protozoans.

TABLE IV.—Seasonal variations in the food of *Notropis bifrenatus* (Cope) based on examination of the stomach. Data plotted in Fig. 1.

Collection	Mar.	Apr.	Apr.	May	Jun.	Jun.	Jul.	Aug.	Oct.
Dates	21*	10	22	17*	21*	26	7	18	24*
No. examined	20	17	21	20	20	11	20	20	32
Item	**Percentages of stomachs containing item**								
Debris	75.0	6.0	19.1	85.0	90.0	9.1	20.0	15.0	93.8
Plant	30.0	0.0	0.0	40.0	50.0	9.1	10.0	25.0	43.8
Insecta	75.0	100.0	90.4	100.0	90.0	91.0	75.0	85.0	31.5
Hydrachnida	35.0	0.0	4.8	5.0	25.0	9.1	15.0	5.0	31.5
Amphipoda	0.0	23.5	42.9	0.0	10.0	72.7	40.0	10.0	0.0
Entomostraca	30.0	70.6	76.2	95.0	65.0	63.6	80.0	80.0	75.0
Item	**The estimated volume of each item given as the percentage of the total volume**								
Debris	38.0	0.1	3.9	27.9	50.1	0.7	3.8	4.1	48.6
Insecta	29.3	86.8	53.3	46.7	34.2	23.5	35.3	48.3	8.9
Amphipoda	0.0	4.4	8.6	0.0	1.8	50.8	24.1	5.0	0.0
Entomostraca	2.6	7.4	24.9	22.8	5.8	16.0	33.1	27.4	23.7
Other food[1]	30.1	1.3	9.3	2.6	8.1	9.0	3.7	15.2	18.8

* Collected in Ithaca, N. Y. For further details compare with dates in Table I.

1 Includes plant materials, hydrachnids, unidentified arthropoda, molluscs, helminth parasites, and protozoans.

Tibbit's Creek, June 17: 70% animal plankton and 30% aquatic insects; five specimens, 23-29 mm. long, from Little Sucker Brook, June 24: 40% animal plankton, 50% aquatic insects, and 10% scuds. Greeley and Greene (1931: 87) and Webster (1941: 161) observed *Notropis bifrenatus* eating the eggs of sunfish. Pate (1933: 153) found, in nine specimens, 28-46 mm. long, from Schroon Lake in Pottersville, New York, taken August 16: 90% aquatic insects, 2% molluscs, and 8% plants. Under the name, Cayuga minnow, Moore (1922: 53, 57) gives the details of the food of thirty-eight specimens. These data are omitted here, for as Adams and Hankinson (1928: 344) point out with good reason, they most likely pertain to the food of both *Notropis bifrenatus* (Cope) and *Notropis h. heterolepis* Eigenmann and Eigenmann.

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Notes on the Amphibians of Venango County, Pennsylvania

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Venango County is situated about midway between Pittsburgh and Erie. Much of its 675 square miles has been glaciated, and the general topography is hilly. The Allegheny River flows through the county, and, with its tributaries, has cut deep valleys in the original plateau. The lowest elevation is about 870 feet above sea level, and the highest point just under 1600 feet. The average last killing frost of the winter falls on May 16, and the first killing frost in the fall, October 3. This makes the average growing season 140 days. The record low temperature for Franklin, the county seat, is –30° F. (although at my home it once unoffiically reached –35° F.) The record high is 106° F. The average annual precipitation amounts to 41.25 inches. The short growing season probably affects the sizes of cold-blooded vertebrates, particularly snakes, as I can think of several species which do not grow as large here as they do in places where the summer is longer, although there may be other factors involved.

Salamanders

1. Mudpuppy—*Necturus maculosus maculosus* (Rafinesque)

Specimens were collected from Sugar Creek and Big Sandy Creek in Venango County. They are not nearly so numerous, apparently, as *Cryptobranchus* in these streams. We used to take many from the outlet of Edinboro Lake in Erie County, with fishing lines baited with worms. In the days when spearing suckers was possible, mudpuppies were observed in abundance in Erie County streams by torch light, but they were carefully avoided, as they were distasteful objects to remove from the spear.

2. Hellbender, Waterdog—*Cryptobranchus alleganiensis* (Daudin)

Since 1932, my brother David and I have caught over 750 hellbenders; about 650 of them from a stretch of Big Sandy Creek about three miles in length. This commercial collecting has apparently diminished their numbers considerably, as they are much more difficult to collect on that stretch of the stream at present. Originally one could find a specimen under almost every suitable rock. In daytime collecting, rocks must be turned over, and as this is rather strenuous work, considering the size of a rock it takes to shelter a specimen, it is easier to catch them at night when they are prowling about the bed of the stream. I believe that we never found two specimens under the same rock. One night the two of us collected 51 in three hours; on another occasion, alone, I caught 36 in two and one half hours. The largest specimen we took measured 22½ inches in total length. In Big Sandy Creek,

specimens rarely grow over 20 inches long. Specimens less than a foot long are never in evidence, a situation that has always puzzled us. They are probably active every month of the year. We have collected them in every month except January, February and March, at which times the creek is usually frozen over or the water is too high and roily.

Their feeding habits probably vary a little with the season, but there is no doubt that crayfish make up a very high percentage of their food. On April 28, 1941, 7 adults regurgitated 17 whole crayfish, many parts of others, 1 helgramite, 1 small sucker, 1 shiner and two other small fishes.

On May 23, 1932 we caught a hellbender that had a 7″ sucker part way down its throat. The sucker was probably dead when picked up. In two days 8 adults regurgitated 35 crayfish and many parts of others.

On June 25, 1932 we dissected 27 hellbenders. None contained any vegetable matter, 3 had empty stomachs, 3 had small stones in their stomachs, 1 had a 4″ sucker, another a 2″ sucker, another a 2″ shiner. 24 each had the remains of from 1 to 5 crayfish. Some hellbenders caught on May 22, 1932, regurgitated crayfish, 60 hours after being caught, that showed very little sign of digestive action. Apparently digestion is slow.

On August 15, 1932, we dissected 5 males and 5 females, and found but few crayfish and little digested matter.

On December 4, 1941 we temporarily placed 4 hellbenders in each of 3 five gallon buckets. The following day two of the buckets contained no regurgitated material, the third contained a sucker about 5″ long, three other small fishes, and two crayfish.

On December 9, 1946, 12 specimens were dissected. 3 contained whole crayfish in their stomachs, 4 had from 1 to 4 crayfish claws in each, and 5 were empty.

From such slight evidence, it would seem possible that more fishes are taken in the cooler months, probably due to greater inactivity of crayfish at that time. Feeding is probably also curtailed in extremely hot weather. Like many other salamanders, however, the hellbender does not seem to be greatly discomfited by near-freezing temperatures. Although I have never taken any from sluggish, warm water streams, they can stand fairly high temperatures for a time at least. On several occasions we have kept them for a month or more in the middle of the summer, in a 55 gallon steel drum with an open top and three or four inches of water in the bottom. This water must have reached a temperature of at least 75° F. for 12 hour periods. The water was changed once or twice a week. I have never observed a hellbender out of water on its own volition, but they will live at least a few days out of water, if kept cool and moist. We ship them in damp sphagnum moss with a fair amount of success.

We have taken hellbenders from Sugar Creek and East Sandy Creek in Venango County and from the Allegheny River in Warren County. I have not taken any from our smaller trout streams. Generally speaking, they seem to prefer streams that are large enough to sustain small-mouth bass, and bass

and hellbenders are there probably for the same reason . . . crayfish are more abundant and more easily caught.

Egg laying takes place about the first of September for this locality. Egg counts from ten females, made on June 25, 1932, were from 350 to 900. It is possible that we counted the eggs for two seasons, however. The eggs ranged in size from less than 1 mm. up to 5 mm. in diameter.

On December 12, 1946, I counted the eggs from five females. One 18 inch specimen contained 245 eggs 2 mm. and over, 87 eggs 1 mm. and under, and 2 large 5 mm. eggs. I suspect that the large eggs were left over from the current season, and that the others represent the supply for two more summers. One other specimen contained 3 large eggs; another, 2. These large eggs were unconnected with the other egg masses. Four of the hellbenders contained from 155 to 455 of the 2 mm. and over sized eggs. The eggs under 1 mm. were not counted.

I know of several persons who have eaten the flesh of the hellbender and pronounced it good. We tried it once, and found it palatable, but quite tough. The toughness may have been due to improper cooking, or to the age and condition of the hellbender, however.

3. RED SPOTTED NEWT, RED EFT—*Triturus viridescens viridescens* Rafinesque

On the night of April 4, 1947, I saw a newt with a small whole worm in its mouth. There were many newts present at the pond. In a 15 gallon aquarium in which I kept some *Natrix sipedon compressicauda,* I introduced a couple of newts in the aquatic stage. The aquarium was divided into half dry area and half water, and was placed upon the window of my bed room. One night I heard several small squeaks from the cage. The following night, upon hearing the sounds again, I arose to investigate. I traced it to one of the newts, which was out of water on a stone. The faint squeaks came in series of two or three each, at intervals of from one to ten minutes apart. It was repeated on at least fifteen different occasions that night. Whether this sound was voluntary, or not, I can't say. The movement of the newt's throat gave the suggestion that it might have been a cough, or caused by other respiratory difficulty, but the impression I received was otherwise. The voice is so faint that it would not ordinarily be heard out of doors, or anywhere where conditions weren't very favorable.

The pond from which I collect most of my aquatic salamanders got very low in the summer of 1946, but while the water was still clear, newts were present in abundance. I collected 60 of them for an experimental worker, two-thirds of which were males. In the early spring of 1947 there were many newts in the pond, once again at its highest stage. In the summer of 1947 the water reached a very low stage in the pond, almost to where there was nothing but mud and algae. I netted a number of sunfish fry, with the intention of saving them to restock the pond later, but got no newts whatsoever. It is possible that they leave the pond when the water becomes too muddy, or, if late in the season, hibernate in the mud.

I have taken a few specimens of the red eft stage in Venango County, usually on forest roads, but they do not seem to be abundant.

4. SPOTTED SALAMANDER—*Ambystoma maculatum* (Shaw)

5. JEFFERSON'S SALAMANDER—*Ambystoma jeffersonianum* (Green)

The pond mentioned in connection with *Triturus* is located in Irwin Township, Venango County, on the farm adjacent to our nursery on State Highway Route 8, about 13 miles south of Franklin. The area of the pond varies from a few square feet in its near-dry stage, to about 4000 square feet when it is completely full in the spring. It is bordered by a few cat-tails, and near by is a clump of trembling aspen. It is surrounded otherwise by fields under cultivation or in meadow, and the nearest wood-lot is about 150 yards away. It was formed by digging limestone for agricultural use. Digging commenced about 100 years ago and continued intermittently up until about 1936. The water was usually siphoned out in the summer to facilitate digging. The greatest depth of the water at its highest stage is about 10 feet. The pond is about 1300 feet above sea level.

The pond became a convenient breeding place for many species of amphibians, as there were no other ponds for several miles. Recent strip mining operations for coal have provided a number of new ponds, the nearest of which is within a quarter of a mile. The strip mining operations are a blot on the landscape, but they will undoubtedly favor amphibian life.

I collected my first *Ambystoma* at this pond in 1931, and have made a great many trips to it since then. In the last 16 years I have walked over one hundred miles from my house to the pond and back, most of the trips on cold, rainy or snowy nights. Most of the trips were made in an effort to answer some of the following questions on *Ambystoma maculatum* and *jeffersonianum:* How early did they arrive at the pond? How long does it take them to reach the pond? From how far do they come? How long do they stay? Do they travel by some instinct or blunder upon the pond by accident? Some years it was impossible for me to be at the pond on the earliest favorable date, and at other times I couldn't visit it frequently enough to establish a probable date when they were last in evidence at the pond.

In 1931 I first visited the pond on April 16 and saw one *maculatum*. On the night of April 17 I observed one of each species; both were about 30 feet from the pond and apparently headed away from it. On April 18 I noticed one *maculatum* close to the pond, going toward it, and finally into it. On the next four nights I saw both species in the pond. On April 22, I caught one *maculatum* which was the last specimen observed. I visited the pond until April 29. The minimum of time that the salamanders could be found at the pond was 6 days for *maculatum* and 5 for *jeffersonianum*, although it was probably much longer.

In 1932 I observed the first *jeffersonianum* on March 30, the first *macu-*

latum on April 1. The eggs of *maculatum* started to hatch on April 21 while adults were still present. On April 25 I saw 6 *maculatum,* two of which were out of the water. I saw one *jeffersonianum* in the water on April 24 and caught another 300 yards from the pond on April 30. The minimum time these species were present in the pond in 1932 was at least 30 days for *jeffersonianum* and 25 for *maculatum.* Of course it must be understood that individual specimens may not remain in the pond for this long a period.

In 1933 I visited the pond on March 6. It was frozen except for a narrow margin at the edge. A shallower portion of the pond was completely thawed out. The nights had been very cold since February 24, and I was at the pond at 7:45 P.M. I saw 7 *jeffersonianum* and caught 2 of them. They could scarcely have reached the pond that night unless they come from the immediate vicinity (a fence row grown up with brush comes close to the pond across a field). If they came from any of the wood lots they must have done so during the night of February 24 or before. I searched the woods, fields, and roads, but could find none traveling. On March 7 I found a *jeffersonianum* about 200 yards away from the pond, apparently heading toward it. It was about 8 P.M. and the air temperature was 39° F. The nights from March 22 to 29 were freezing, and unsuitable for amphibian travel. On the 30, I caught 7 *maculatum,* four of them in puddles away from the pond, one of which was about 300 yards away. On April 3 I saw 2 *maculatum* in a puddle in a rut made by a car, apparently headed away from the pond. There were several *jeffersonianum* observed in the pond. In 1933 it is probable that *jeffersonianum* was in the pond from February 24 until April 3, or 39 days, *maculatum* for at least 20 days. The last *maculatum* was observed, out of the pond, on April 5.

In 1934, incomplete records show *jeffersonianum* to have been in the pond for a minimum of 7 days, *maculatum* for 12 days.

In 1940, the first favorable night for migration to the pond was on March 29. I saw the first *jeffersonianum* on March 30, two specimens, which probably couldn't have come far. I observed the first *maculatum* on April 7, and noted none subsequently.

In 1941, the first suitable night was that of March 31, when I observed four male *maculatum* and three males and three females of *jeffersonianum.* All of them were found on the dirt road, or the ditches bordering it, that separates the nearest woods from the field containing the pond, about 150 yards distant from the pond. I made a mark on the road near one specimen of each species, and then continued hunting. I left them undisturbed for ten minutes, then returned to find that the *maculatum* had not moved at all, and the *jeffersonianum* a distance of only two feet. On May 7 occurred the first rain since a drizzle on March 31. I caught one *maculatum* from the pond. The latter species must have been in the pond for at least 37 days.

In 1942 the first possible night for migration occurred on March 16. I then

observed two *jeffersonianum* on the above mentioned road, and seven in the pond; also one *maculatum* in the pond. Of the *jeffersonianum* five were males and four females. On the night of April 3 I observed both species in the pond. Both were present in 1942 for at least 18 days.

In 1946 I visited the pond on the first thawing nights of the spring, March 4 and 5. On the 5th I saw 3 *jeffersonianum* in deep water. On March 8, I saw 4 *maculatum* in the pond and 1 emerging from the woods 150 yards away. I caught 7 *jeffersonianum* and saw many more. March 8 was the first rainy night, preceded by several warm nights.

The first favorable night in 1947 occurred on March 24. The pond was still frozen except for a narrow margin of a couple of inches along its perimeter. I caught a *jeffersonianum* on top of the barely wet ice, 18 inches from shore. I observed specimens on April 4, 7, and 11. By the 20th they were apparently gone.

On January 1, 1948, occurred a thunderstorm with copious rainfall. The ground had previously been frozen sufficiently to imprison any salamanders wherever they might be. Thawing on December 31 and January 1 was sufficient to release them for migration if they were so inclined. The pond was only half full from the previous summer's drying out, and there was about three inches of water over fairly thick ice. No animal life of any kind was observed. It is probable that *Ambystoma* must hibernate a certain length of time before instinct calls them to renew their activities.

I have never observed adults of either species in the daytime, in or out of water. Late in the summer of 1932 I saw a larval *Ambystoma* eating a small tadpole. In 1934 I kept a specimen of maculatum in a fish bowl containing sand on the bottom, with enough water to cover the salamander's body. The temperature was fairly uniform from 65-70° F. The specimen was caught on April 10, and died on August 5, living for 117 days without any food. I have kept both species alive for periods of a year, by putting them in a five gallon bucket with damp sphagnum moss, kept in a cool dark place. When it occurred to me, I threw in a few small worms, sometimes months apart. These were apparently eaten, as when none were introduced for long periods, the salamanders became emaciated, but regained plumpness when sufficient worms were supplied.

On the night of March 21, 1933 the temperature became as low as 24° F. I was keeping 10 *jeffersonianum* and 3 *maculatum* in a wash tub, temporarily, in about an inch of water. I forgot to take them indoors overnight, and they became frozen in the ice, the water freezing almost solidly to the bottom of the tub. I let them thaw out very gradually. They were held in the ice for five hours, after which five of the *jeffersonianum* were dead, and all the rest living.

I once placed some *Ambystomas* in an aquarium with some flat-tailed water snakes (*Natrix s. compressicauda*). The snakes ate two *jeffersonianum* but the *maculatum* they would not eat, although several grasped the specimens in their mouths temporarily.

To summarize on some of the habits of *maculatum* and *jeffersonianum*, after a period of hibernation, when the ground is sufficiently thawed out in the spring, they head toward a pond, probably the first night that the air temperature is above freezing. This usually occurs during a rain, perhaps incidentally, as rains are common with above freezing temperatures in February and early March. I have seen them traveling on nights when it wasn't raining. They remain in the pond for an indefinite period, that may be over a month, as a species. As individuals the time spent at the pond is possibly much less. When leaving the pond they are probably more particular about choosing a rainy night, as it is later in the season, and the vegetation is likely to be dried out so that they can't keep moist. It is probable that the specimens reaching a pond first leave it earlier than those that reach it later. I have noticed no difference in the arrival or departure dates between the sexes. I believe that when they are ready to leave, the salamanders crawl out of the water a little ways, and if it is too dry, or too cold, they return.

It is doubtful whether they travel any great distance to the pond. All of the specimens I have seen out of water travel very slowly, and the fact that they may be found at a pond so very soon after it is physically possible for them to travel, indicates that at least the early arrivals must have hibernated close to the pond. I have examined the pond many times in the late fall and early winter in an attempt to ascertain if any *Ambystoma* spent the winter in the pond, but I have never seen them then. (Incidentally, I have never taken *Ambystoma opacum* in Venango County.)

It is probable that some instinct prompts them to the direction they are to travel, and that they do not find the pond by accidental wandering. The fact that they are so very rarely seen anywhere in the open except in the vicinity of a pond, and that they may be observed in such numbers in the pond, would discount accidental discovery. Only once have I found a specimen in the summer. It was a *jeffersonianum* and was found in a rotten log about 300 yards from the pond, in our woods. From the numbers observed at the pond in the spring, and from the deduction that they can't travel great distances in the short space of time they have in which to reach it, it seems odd that they are not more frequently met with in the summer, particularly when one searches, as I have, over much presumably favorable habitat such as rotten logs and stumps, slab piles, sawdust heaps, and other litter.

6. DUSKY SALAMANDER—*Desmognathus fuscus fuscus* (Rafinesque)

This species is fairly common in Venango County.

7. MOUNTAIN SALAMANDER—*Desmognathus fuscus ochrophaeus* Cope

I have taken more specimens of this than the former in Venango County.

8. RED-BACKED SALAMANDER—*Plethodon cinereus cinereus* (Green)

This species is very common, and may readily be collected anywhere in forest land, under rocks, fallen trees, and other similar cover. I have also collected one in sphagnum moss in association with *Hemidactylium*.

9. SLIMY SALAMANDER—*Plethodon glutinosus glutinosus* (Green)
The slimy salamander is sometimes called the spotted salamander by the uninitiated. I have collected some from forest land, and caught one in our own basement.

10. FOUR-TOED SALAMANDER—*Hemidactylium scutatum* (Schlegel)

One specimen was taken from under a slab on a woods road near South Sandy Creek, and another from sphagnum moss near Scrubgrass Creek.

11. PURPLE SALAMANDER— *Gyrinophilus porphyriticus porphyriticus* (Green)

I have taken this species from springs and small runs, and one specimen from sphagnum moss. On April 15, 1939 I was fishing in Dennisons' Run for trout. On a small hook baited with a worm, I was surprised to catch a large specimen from rather swift water.

12. RED SALAMANDER—*Pseudotriton ruber ruber* (Sonnini)

I have taken a number of specimens, both larval and adults, from Venango County. On July 26, 1939 I caught one while it was crossing the concrete highway between Pearl and Bullion, in the rain.

13. TWO-LINED SALAMANDER—*Eurycea bislineata bislineata* (Green)
I have collected a few specimens from the county.

14. LONG-TAILED SALAMANDER—*Eurycea longicauda longicauda* (Green)

This species is probably a little more common than the preceding. I have made no effort to collect extensively any of the *Plethodontidae*.

FROGS AND TOADS

The following notes on frogs and toads consist mostly of dates when each species were first taken for the year mentioned. In most instances, the date given was the earliest that the call of the species was noted; in a few cases they were taken on that date but may have been present earlier. For *Bufo*, practically all of the dates represent the first night they were heard calling in this vicinity. A short distance farther south, frogs emerge a few days earlier, and in the latitude of Pittsburgh, about 60 miles south, they may emerge from one to two weeks earlier.

15. AMERICAN TOAD—*Bufo terrestris americanus* Holbrook

First date calls were heard: April 25, 1932; April 10, 1933; April 27, 1935; April 28, 1940; April 12, 1941; April 15, 1942; April 5, 1947. Dates of the last nights of calling are much more difficult to record. In 1940 the last call was noted about May 26. The same date was recorded for 1941. In another pond, near Bullion, my brother and I collected 77 specimens in thirty minutes

on May 1, 1940. They are never very numerous in the pond near my home. The habitat is probably not as suitable.

On August 23, and again August 24, 1946, I caught immature *americanus* that measured 8 mm. in length, about a quarter of a mile from the nearest pond.

16. EASTERN CHORUS FROG—*Pseudacris nigrita triseriata* (Wied)

First dates collected or heard: March 30, 1932; March 31, 1933; April 9, 1934; April 4, 1940; March 31, 1941; March 17, 1942; March 8, 1946; April 4, 1947. On March 8, 1946 a specimen was caught crossing the highway in the rain, before any were heard calling at the pond. There were no species of frogs present at the pond up until that date. In 1934 *Pseudacris* was more common at the pond than *Hyla c. crucifer*. Before then, and more particularly since then, they have been decidedly fewer than *crucifer*.

17. SPRING PEEPER—*Hyla crucifer crucifer* Wied

First dates: April 16, 1931; March 30, 1932; March 30, 1933; April 2, 1934; April 6, 1940; March 31, 1941; March 17, 1942; April 4, 1947. In 1934 they quit calling about the first of May. In 1933 they started to scatter from the pond about May 2, although some were still calling in the vicinity. On April 13, 1933 one could hear feeble calls when the air temperature was 30° F. On April 22 of that year, the weather became cold, and there was an inch of snowfall on the ground. The temperature was 32° and it was snowing and raining at the same time. The *Hylas* were sitting out of the water, but their calls were feeble and infrequent.

18. BIG TREE FROG—*Hyla versicolor versicolor* LeConte

First dates: May 27, 1931; May 22, 1933; May 26, 1941; May 24, 1940.

19. BULL FROG—*Rana catesbeiana* Shaw

Bull frogs were never found in the pond near my home. They are present in most of the larger streams of the county, and I have collected them from the Allegheny River and Big Sandy Creek. On August 10, 1932, at Milton, Pennsylvania, I saw a house cat carrying a frog in its mouth, either of this or the following species.

20. GREEN FROG—*Rana clamitans* Latreille

My notes on this species are scant. I have found them at the pond as early, at least, as April 19, and they are present in the pond throughout the summer as long as any water remains. If they hibernate in the pond, they emerge rather late, as they are not ordinarily seen early in the spring.

21. PICKEREL FROG—*Rana palustris* LeConte

On April 19, 1931, pickerel frogs were observed in great numbers en route to the pond. They were still present on May 25. Adults are not found at this pond in the summer months, but newly transformed specimens are very numerous. First dates observed: March 30, 1932; April 3, 1933; April 9, 1934; April 6, 1940; April 3, 1942; April 11, 1947.

22. LEOPARD FROG—*Rana pipiens* Schreber

This species is rare at the pond, and throughout this part of Venango County it is much less common than *palustris*. I caught an immature specimen at the pond in 1932, and an adult on April 7, 1940.

23. WOOD FROG—*Rana sylvatica sylvatica* LeConte

First dates: April ?, 1931; April 2, 1932; April 5, 1933; April 7, 1940; March 31, 1941; March 16, 1942; March 8, 1946; April 5, 1947. On March 8, 1946 a specimen was caught half way between the woods and the pond. *Sylvatica* appears to stay at the pond a much shorter period than the other species.

American Caudata. III.

The Status of Pseudotriton montanus in Ohio[1]

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The presence of *Pseudotriton montanus* ssp. in Ohio was first reported by Walker (1933: 224), on the basis of specimens in the Ohio State Museum. Subsequently, Bishop (1941: 14) described *Pseudotriton montanus diastictus* from Carter County, Kentucky, and various West Virginia stations, and later extended the range of this race to include southern Ohio to the exclusion of other forms of *P. montanus* (1943: 386; map 45). As matters stand today, most workers follow Bishop in considering *diastictus* the only representative of *Pseudotriton montanus* in Ohio.

That the salamanders of southern Ohio do not fit readily into any arrangement predicated largely on specimens from adjacent areas is not surprising. The region is poorly represented in most herpetological collections, although it has been an increasingly fruitful source of novelties within the past fifteen years, yielding such unusual and important records as: the allotype and many paratypes of *Plethodon richmondi* (Netting and Mittleman, 1938), the type of *Gyrinophilus porphyriticus inagnoscus* (Mittleman, 1942), and the only known Ohio specimens of *Plethodon wehrlei* (Walker, 1933), *Leiolopisma unicolor* (Stupka, 1933), and *Scaphiopus h. holbrookii* (Gier, 1945).

This general region forms the northern and western range limit of many caudate genera and species, such as *Gyrinophilus, Pseudotriton, Aneides aeneus,* and *Plethodon richmondi.* These terminal populations are often perceptibly different from others within the range of their species, although they are not always recognizable taxonomically. Taxonomic treatments of groups including forms occurring in this region are likely not to be consonant with local conditions, unless specific attention has been given to specimens collected in southern Ohio. The status of *Pseudotriton montanus* ssp. is an excellent case in point.

Our collecting experience in southern Ohio has produced many specimens of *montanus* thoroughly distinct from *diastictus,* at least as compared with large series of topotypic specimens from northern Kentucky, but we have seen and collected other specimens indistinguishable from *diastictus.* In an effort to determine the status of these Ohio specimens, we have reviewed the extant

1 Contribution Number 261 from the Department of Zoology, Kansas State College, Manhattan, Kansas. The field work and part of the comparative study were done while the authors were at Ohio University, Athens, Ohio. Acknowledgements for gifts of specimens, loans, and many other courtesies, is gratefully accorded to Messrs. John Hughes, Ralph Dury, and Edward S. Thomas, and to Drs. Doris M. Cochran and W. C. Stehr.

Plate I. *Pseudotriton m. montanus* Baird. Cotype, USNM 3839. South Mountain, Carlisle, Pa. Preserved specimen, natural size. Photographs kindly provided by Dr. Doris M. Cochran.

material, and have compared it at length with types and with other specimens.

Baird's original description of *Pseudotriton montanus* (1849: 293) was based on two specimens[2] taken "on South Mountain near Carlisle, Pennsylvania" (Plate I). Since Baird's publication, no additional specimens have been found in Pennsylvania, the majority of known specimens originating in the Appalachian Highlands from Maryland to Georgia. Exhaustive study and detailed comparisons of the types with other *montanus,* and even with other species of *Pseudotriton,* lead us to the conclusion that only color and pattern will distinguish *montanus* from other forms of its genus. No statistically significant mensural or proportional difference are detectable. Dental character-

2 The original description mentions two specimens, one six inches long and the other three. Baird's type is now USNM 3839, and catalogued under this number and included in the same jar are two metamorphosed adults—undoubtedly those described by Baird—and one very large larva.

istics are quite variable, and hence unreliable—many *ruber* as well as *diastictus* perfectly duplicate Baird's types in this respect. Baird's *montanus* has been well summarized by Bishop (1943: 377), who describes it as a large *Pseudotriton* with "Ground color above dull, light to dark chocolate-brown; dorsal and lateral dark spots usually distinct, sometimes obscure in old individuals, but when present extending well onto lower sides; venter with at least a few, often many, small, brown, widely separated dots; iris of eye dark brown. . . ." Specimens that we consider "typical" *montanus*, on the basis of direct comparison with the types, are:

MARYLAND. *Anne Arundel County: Priest's Bridge* (USNM[3] 101427-8). NORTH CAROLINA. *Forsythe County:* Salem (USNM 3848—two specimens). *Orange County:* Hillsboro (USNM 7031—two specimens). *Wake County:* Raleigh (USNM 16631, 22174, 38236-7, 49681, 57182-5). SOUTH CAROLINA. (*Anderson County:* Anderson (USNM 3873—five specimens). VIRGINIA. *Fairfax County:* George Washington Spring, 0.5 mi. S. of Mt. Vernon (USNM 73603-10).

The enumerated specimens provide a representative series of *montanus*, and facilitate comparisons involving color and pattern. By direct comparison we find that specimens from northern Kentucky (Carter, Kenton, and Madison counties) are, as a group, usually distinguishable from Baird's types, and from Maryland, Virginia, and Carolina specimens. Bishop (1943: 388) has characterized these Kentucky specimens by stressing their relatively large and few spots, their light ground color, and their usually immaculate sides below the level of the insertions of the limbs. Yet, these differences are by no means definitive, for they are often reproduced in eastern *montanus* to a greater or lesser extent. For example, OUZ A-616, from Raleigh, N. C. (Pl. II., fig. 1), has rather large dots, a comparatively light ground color, and nearly immaculate sides below the dorsolateral line. This specimen is not a *diastictus* of course, but the difference between it and some topotypes of Bishop's race is very finely qualitative. Similarly, the "normal" variation in Kentucky *diastictus* is considerable, from pale salmon color and a very few small dots (Pls. II. and III., fig. 11), to clear red and heavy dotting. The lack of lateral spotting, and the unclouded dorsum, is quite constant in the Kentucky *diastictus* we have seen.

With "typical" Kentucky *diastictus* on the one hand, and "typical" *montanus* on the other, it is not difficult to identify most Ohio specimens of this species. Thus, OSM A-981 and 673 (Liberty Twp., Jackson County) and OSM A-92-54, 55 (Green Twp., Adams County), are, or are very close to, *diastictus*, so far as a light ground color, large and widely separated dots, and sparse lateral spotting constitute identifying criteria (see Pls. II. and III., figs. 9-10). On the other hand, OSM A406-1 (Washington Twp., Jackson County), OSM 883.5, 919.1, 919.2 (Gallia County), OUZ A-74, OSM 294.18 (Liberty Twp., Jackson County), and OUZ A-286 (Carbondale, Athens County), are indistinguishable from eastern *montanus* on the basis of direct comparison with Baird's types and other eastern specimens enumerated heretofore (Pls. II. and III., figs. 2-6). A third form, which is approximately intermediate between

[3] Abbreviations for collections: USNM = United States National Museum; OSM = Ohio State Museum; OUZ = Ohio University, Department of Zoology; CMNH = Cincinnati Museum of Natural History.

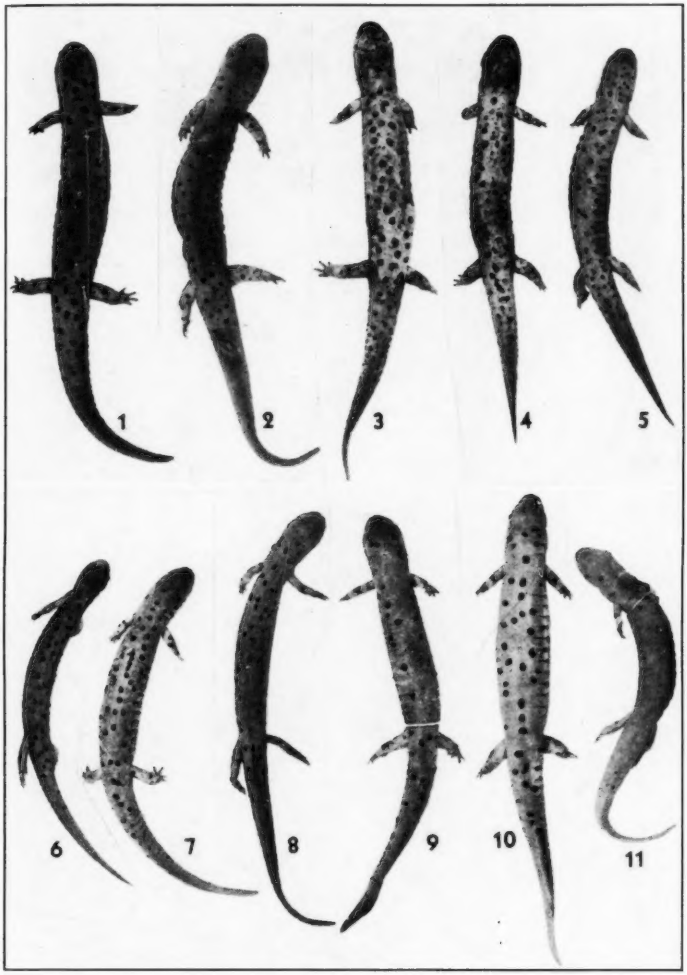

Plate II. *Pseudotriton montanus* ssp. Figs. 1-6.—*P. m. montanus*.—1. OUZ A-616. Raleigh, N. C.; 2. OUZ A-74. Liberty Twp., Jackson Co., O.; 3. OSM 919-1. Gallia Co., O.; OSM 919-2. Gallia Co., O.; 5. OSM 883.5. Gallia Co., O.; 6. OUZ A-286. Carbondale, Athens Co., O. Fig. 7.—*P. m. montanus* x *diasticus*. OSM 980. Liberty Twp., Jackson Co., O. Fig. 8.—*P. m. montanus* OSM 406-1. Washington Twp., Jackson Co., O. Figs. 9-11—*P. m. diasticus*.—9. OSM A-92-54. Scioto Co., O.; 10. OSM 981. Liberty Twp., Jackson Co., O. 11. CMNH 1831. Carter Co., Ky. Dorsal views, one-half natural size.

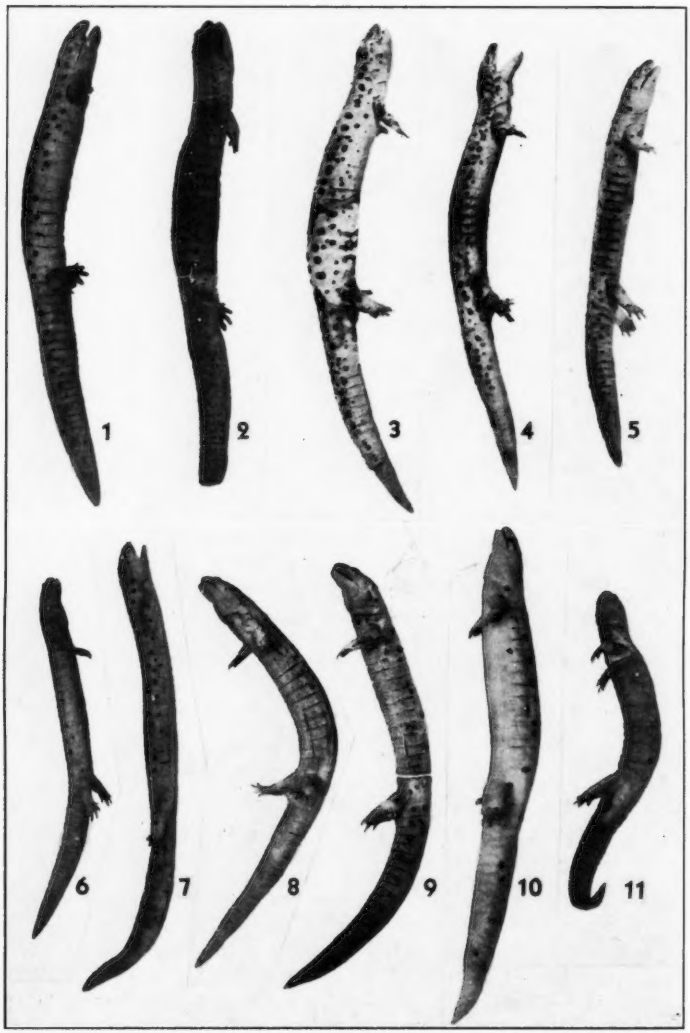

Plate III. *Pseudotriton montanus* ssp. All figures the same as in Plate II. Lateral views, one-half natural size.

montanus and *diastictus* is well represented by OSM 979, 980 (Liberty Twp., Jackson County), OSM A-973 (Ross County), and OSM 449 (Scioto County); such specimens have the well-developed dots and nearly immaculate sides of *diastictus*, but are similar to *montanus* in their ground color and number of dots (Pls. II. and III., fig. 7).

To summarize the situation with respect to Ohio specimens: some individuals from Jackson and Adams counties are identifiable with *diastictus;* the majority of specimens from Jackson County, and all the specimens available from Gallia and Athens Counties, are not distinguishable from *montanus;* intermediate specimens are known from Jackson, Ross, and Scioto counties. A single locality, White's Gulch, Liberty Township, Jackson County, has produced both pattern extremes, as well as intermediates. It appears then, that *diastictus* is found at least in Adams County, while *montanus* occurs in the extreme southeastern corner of the state (Athens and Gallia counties, and by inference, Meigs and Washington counties). The two races meet and intergrade in the Scioto River valley, in Ross, Jackson, Scioto, and probably Lawrence counties.

The occurrence of *montanus* in southern Ohio poses a zoogeographical problem; the populations of adjacent Kentucky and West Virginia[4] being distinguished as *diastictus*, it is not easy to explain the presence of *montanus* in Ohio. Perhaps the hypothesis that Ohio *montanus* constitute a relict population persisting from the original trans-Alleghanian *montanus* or *montanus*-like stock fits the known facts as well as any other, but this still offers no explanation for the differentiation of *diastictus* in northern Kentucky and extreme southern Ohio, and the static nature of the *montanus* population of Athens and Gallia counties. The recognition of *montanus* in Ohio requires the acceptance of a discontinuous range for this race, or the postulation that there is an unknown complement of *montanus* across southern West Virginia; only extensive collecting in the region in question can settle this point.

4 Bishop (1943: 386) includes northern and eastern Tennessee in the range of *diastictus;* we have seen only two specimens from Tennessee (USNM 3870, Tyree Springs, Sumner County), and these we cannot distinguish from other *montanus*.

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Notes on a Herpetological Collection from the Middle Mississippi Valley

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The herpetological fauna of the region within a hundred mile radius of the confluence of the Mississippi and Ohio Rivers is especially rich both in number of species and number of individuals. Here southern forms ranging up the valley of the Mississippi come in contact with closely-related northern and western subspecies to produce many interesting variants. This is truly a land of challenges and surprises for the herpetologist.

During the summer of 1946, the writers made three trips into the southwestern part of Illinois, collecting chiefly in Jackson, Union, and Alexander Counties. In the summer of 1947, we again visited this area and continued southwestward into Missouri and the northeast corner of Arkansas. We recorded 38 species of reptiles and 18 species of amphibians and were able to obtain preserved specimens of most of the forms seen.

The so-called Illinois Ozark region of western Union and Jackson Counties has long been a favorite collecting area for herpetologists. For a detailed study of the topography and herpetofauna of this region, the reader is referred to the admirable paper of Cagle (1942:164-200). Additional references will be found in the bibliography. In brief, this country is characterized by moderately high, deeply dissected sandstone ridges terminating along the valley of the Big Muddy River in precipitous bluffs. The lowgrounds are swampy and subject to frequent flooding. Wolf Lake, which will be referred to frequently in the following pages, is located in the western part of Union County and extends from the town of Wolf Lake north almost to the point where the Big Muddy turns west before emptying into the Mississippi. The lake is very narrow, and the patches of open water are rarely as much as fifty yards in width. Its east bank closely parallels the rocky ridge that forms a southward extension of the bluffs of the Big Muddy. On the west, it is bordered by a belt of dense hardwood swamp, some of which is fairly dry during the summer. The lake is apparently spring-fed and, according to residents, maintains a fairly constant level. The lowgrounds teem with frogs; while the rock ledge-swamp association is very favorable to certain snakes, especially the cottonmouth, *Agkistrodon piscivorus*.

The eastern Missouri Ozark country of Wayne County is higher and drier than that of southern Illinois. Altitudes in excess of 1220 feet are reached on some of the ridges. The uplands are wooded and rocky; high cliffs border the larger streams. The streams themselves are clear and spring-fed with little or no marginal swamp.

The river swamp, typical of the larger southern stream valleys, has its beginnings in the lowgrounds of extreme southwestern Indiana. It is charac-

terized by shallow lakes and bayous bordered by dense swamp. The predominant trees are the bald cypress (*Taxodium distichum*), the pecan (*Hicoria pecan*), and the water hickory (*Hicoria aquatica*). This sort of swamp is seen spottily through the southern tip of Illinois and begins to attain its full development in the extreme southeast extension of Missouri and adjacent portions of Arkansas, Kentucky, and Tennessee. Much of the swampland has been greatly modified by drainage and agriculture. In some places the trees remain, but the water has been reduced to scattered mudholes; in others the land has been cleared and cultivated, the only permanent water remaining being in drainage canals and roadside ditches.

We found that the early morning and late afternoon hours were best for collecting terrestrial reptiles. The hot midday hours were used for traveling or for making camp, preserving specimens, and writing field notes. Night collecting on foot using hand flashlights and a Coleman gasoline lantern was the method of choice for obtaining frogs and some aquatic snakes. Automobile collecting—driving slowly along little traveled roads with a man perched on the fender—was tried on a number of occasions but without conspicuous success. A boat was almost essential for penetrating the river swamps and cypress lakes. Basking watersnakes and turtles could often be approached in this manner and secured by hand or with a dipnet. Some watersnakes could best be taken at their basking places by slowly wading or swimming in toward them. Naturally this approach could only be used if the harmless nature of the snake had been determined. A number of interesting specimens, especially snakes, were found dead on the road (DOR) as we drove from one collecting site to another. On most of our trips we were accompanied by Mr. Frank Powell and Mr. Harry Carnighan, both experienced and resourceful reptile collectors. In some cases, local residents aided us considerably in locating good collecting sites.

An annotated list of the reptiles and amphibians observed follows. Preserved examples of most of these are in the senior author's collection or in the collection of the University of Michigan Museum of Zoology. Wherever possible, the popular name used is that applied to the animal in the locality where it was collected.

Reptiles

TURTLES

Chelydra serpentina serpentina (Linnaeus). Snapping turtle.—We collected a living specimen 3 miles east of Murphysboro, Illinois and saw numerous dead specimens in the river swamp regions of southern Illinois, southeastern Missouri, and northeastern Arkansas. It seems to be common in lakes, swamps, and muddy streams throughout the area under consideration.

Sternotherus odoratus (Latreille). Musk turtle.—We collected specimens in shallow, muddy water at Wolf Lake, Illinois and Hovey Lake, Posey County, Indiana. In Alexander County, Illinois and Greene County, Arkansas we saw several specimens killed by fishermen. This turtle also is probably a common inhabitant of muddy ditches, lakes, and streams.

Kinosternon subrubrum subrubrum (Lacépède). Mud turtle.—In a rela-

tively dry portion of a swamp near Cardwell, Dunklin County, Missouri, we found three of these turtles partially buried in the mud. At Big Lake National Wildlife Refuge, Arkansas, 6 eggs presumably of this species were found under a log at the edge of a drainage canal.

Terrapene carolina carolina (Linnaeus). Box turtle, dry-land terrapin.—These turtles were very common in the forested hills of Jackson County, Illinois. Following a rainy night, at least a score were seen wandering in woods and pastures. Several were feeding on earthworms and fungi. Few were seen in the swampy lowgrounds of Union County, Illinois; and none were found near the cypress lakes.

Terrapene triunguis (Agassiz). Dry-land terrapin.—Many of these turtles were seen, living and dead, on the roads between Cape Girardeau and Lodi, Missouri. Specimens were collected at Sam A. Baker State Park, Missouri. This seems to be an upland species generally found in drier situations than *T. carolina*.

Chrysemys picta dorsalis (Agassiz). Pond turtle.—These turtles were common throughout the southern tip of Illinois in roadside ponds and ditches as well as in the larger lakes and swamps. We collected specimens only at Horseshoe Lake, Alexander County. They were quite typical of the subspecies *dorsalis;* but specimens tending toward the subspecies *marginata* and *bellii* are common in southern Illinois. *Chrysemys* inhabit shallow water where there is much aquatic vegetation, and seems less wary than the other aquatic turtles.

Graptemys pseudogeographica pseudogeographica (Gray). Map turtle, hardshell turtle.—A few turtles seen along the Big Muddy River in Union County, Illinois were identified as this species but could not be collected. An

Fig. 1.—Cypress lake in northeastern Arkansas: a favorite basking place for turtles and large watersnakes.

immature specimen was collected in a drainage canal at Big Lake National Wildlife Refuge, Arkansas.

Pseudemys scripta elegans (Holbrook). Hardshell, turtle, cooter.—We saw more of these turtles than any other species. They were seen throughout the area studied, usually as they were basking on logs and stumps, in swamps, canals, and cypress lakes. During May and early June, many large examples were found crushed on roads. Nearly all of these were egg-containing females. We examined several melanistic specimens—all adult males. We collected several specimens but lack of suitable containers severely restricted the number of large turtles we were able to preserve in the field.

On June 14 in Union County, Illinois just before sunset, we saw a large *Pseudemys* digging her nest in the side of a levee about fifty feet from the water. She scooped out the soil with alternate rotary motions of her hind feet. Returning to the spot thirty minutes later, we found the nest abandoned and no eggs laid. The animal had excavated a cavity about two inches in diameter and equally deep.

Trionyx spinifera spinifera (LeSueur). Softshell turtle.—One immature specimen was collected in the St. Francis River near Lodi, Missouri; and several others were seen in the same locality. It inhabits large bodies of water and is shy and strictly aquatic. The species is probably much more common and widespread in this area than our observations indicate.

LIZARDS

Sceloporus undulatus hyacinthinus (Green). Fence lizard.—Fairly numerous on the dry, timbered ridges of the Illinois and Missouri Ozarks. Usually found close to the ground on fallen trees or rock piles, it climbs trees readily when pursued.

Eumeces fasciatus (Linnaeus). Blue-tailed lizard, scorpion, five-lined skink.—Observed in all localities visited. It frequents rather damp, shady situations and may be found in the open or under logs, boards, and rocks. We saw many in the river swamps sunning on piles of driftwood or limbs overhanging the water. One was basking on a huge cypress log floating about a hundred yards from shore. In no case did the lizard take to the water to escape. In the hilly regions we found specimens on wooded, rocky slopes. We saw all color phases from the bright blue-tailed juveniles to old red-headed males.

Eumeces laticeps (Schneider). Giant skink, scorpion.—A very large skink seen high in a tree in Jackson County, Illinois was identified as this species because of its size. It escaped. Juveniles of this lizard may have been identified in the field as the preceding species.

Eumeces anthracinus (Baird). Coal skink.—A specimen was collected under a log at the edge of a field near the St. Francis River in Wayne County, Missouri on June 13. It was curled about a clutch of 12 eggs but made no effort to defend them. About thirty hours after this lizard had been captured, we returned to the nest and found most of the eggs destroyed by ants.

Leiolopisma laterale (Say). Ground lizard.—Specimens were taken in a variety of habitats—from the dry, rocky ridges of the Ozarks to sandy sections of the river swamps where the soil was quite damp. All specimens were found on the ground, usually under logs, rocks, and rubbish. A single egg, presumably laid by one of these little lizards, was found beneath a stone on a dry hillside in Wayne County, Missouri on June 13. The shell of this egg was very thin and rather brittle, reminding one of the eggs of tropical geckos.

Cnemidophorus sexlineatus (Linnaeus). Racerunner, fieldstreak.—We saw several of these lizards along the edges of dry, sunny fields at Sam A. Baker State Park, Missouri but were unable to collect a specimen. This is a terrestrial species that takes refuge in burrows when pursued.

SNAKES

Carphophis amoena vermis (Kennicott). Worm snake.—One specimen was collected under a rock on a wooded hillside west of Lodi, Wayne County, Missouri.

Diadophis punctatus arnyi (Kennicott). Ring-necked snake.—Three specimens were collected under rocks on wooded ridges in Wayne County, Missouri. When handled or disturbed, these little snakes curl the tail into a spiral elevating it in such a way as to show the bright pink underside. We have not observed this habit in other subspecies of *punctatus*.

A peculiar specimen of ring-necked snake collected at Pine Hills Recreation Area, Union County, Illinois seems to be intermediate between the subspecies *arnyi* and *stictogenys*. It is an adult male with 15 scale rows, 160

Fig. 2.—The Ozarks of Wayne County, Missouri. Terrestrial reptiles such as *Cnemidophorus*, *Coluber*, *Masticophis*, *Sistrurus miliarius*, and *Terrapene triunguis* predominate here.

ventrals, and 42 caudals. In life the belly was bright orange-yellow marked with black spots which tend to form a single median row.

Farancia abacura reinwardtii (Schlegel). Stinging snake.—Two specimens of this large and showy snake were found dead on the road near Neelyville, Butler County, Missouri. The surrounding country was typical southern farm land with shallow weed-choked ditches on either side of the road. One of these snakes had a small *Siren* in its throat. The shed skin of another *Farancia* was found under a rotten cypress log in a nearly dry swamp in Green County, Arkansas.

Heterodon contortrix contortrix (Linnaeus). Blowing viper, spreadhead, hog-nosed snake.—We collected only two of these snakes—an adult in a pasture 4 miles west of Murphysboro, Illinois and a newly-hatched individual on a sandy road near Horseshoe Lake, Alexander County, Illinois. DOR specimens were seen in Posey County, Indiana and Bollinger County, Missouri.

Opheodrys aestivus (Linnaeus). Green snake.—A small specimen was collected in a thicket on a hillside 4 miles west of Murphysboro, Illinois; and another was found DOR in the same region. They were reported by residents at various points in the Illinois and Missouri Ozarks, but the species does not seem to be plentiful.

Coluber constrictor flaviventris Say. Blue Racer.—In southern Illinois (Jackson, Union, Alexander, and Williamson Counties); Wayne County, Missouri; and Greene County, Arkansas, we saw specimens of the blue racer along roads and in fields and dry woods. They are active animals, difficult to capture in the open unless taken by surprise. One adult reptile had its stomach packed with insect remains; while a large grasshopper was removed from the stomach of a young individual. These snakes are black or very dark grey above; bluish-white below. They appear closer to the subspecies *flaviventris* than to *constrictor* but are not typical of either form.

Masticophis flagellum flagellum (Shaw). Coachwhip snake.—A fine specimen of this reptile, 66 inches in length and lacking part of its tail, was collected in brush near one of the public camp sites at Sam A. Baker State Park, Wayne County, Missouri.

Elaphe obsoleta (Say). Chicken snake.—A common snake in the Illinois Ozarks, this species was taken in all localities visited. Specimens were collected in trees, on the ground, and beneath logs and boards. Numerous examples were found crushed on highways. A forest habitat is preferred. This snake attains a large size. One 68 inches long was collected near Murphysboro, Illinois; and two specimens, each slightly over five feet, were found under a log on a levee in the swamps of Dunklin County, Missouri. One of these reptiles disgorged the shell membranes of several eggs about the size of hen eggs.

Elaphe obsoleta in this part of its range shows considerable variation in color and pattern. Most adult snakes are dark brown with a fairly distinct blotched pattern; however we saw almost uniformly black specimens near Wolf

Lake, Illinois and Cape Girardeau, Missouri. Adults with a strongly blotched pattern such as is seen in the subspecies *confinis* were seen in White County, Illinois, Posey County, Indiana, and Mississippi County, Arkansas.

Lampropeltis getulus holbrooki (Stejneger). Speckled king snake.—We collected one specimen on a wooded hillside 5 miles west of Murphysboro, Illinois and another on a levee in the river swamp of Mississippi County, Arkansas. Near Lutesville in the Missouri Ozarks, we found another specimen DOR.

Lampropeltis getulus nigra (Yarrow). Black king snake.—Two of these snakes were taken along the margin of a swampy woods near Grimsby, Jackson County, Illinois. The larger was almost uniformly black with light centers on a few of the lateral scales; the smaller showed a trace of narrow light crossbands. Both appeared identical with specimens of *nigra* from southern Indiana. It is probable that Jackson County is in the area of intergradation between *nigra* and *holbrooki*.

Lampropeltis triangulum syspila (Cope). Red milk snake.—All of our specimens were collected June 15 at Pine Hills Recreation Area, Union County, Illinois. The first was found under a flat stone near a creek; the second at the edge of a road on a high ridge; and the third on a swamp road after dark. Lizard eggs were found in the stomachs of two of these snakes.

Natrix sipedon sipedon (Linnaeus). Common watersnake.—Specimens collected along Big Creek and the St. Francis River in Wayne County, Missouri are referred to this subspecies; although further collecting in this area

Fig. 3.—A partially drained cypress swamp in Alexander County, Illinois. This is a typical habitat for various fossorial reptiles and amphibians as well as some species of *Natrix*.

would probably show that the population is intermediate between *sipedon* and *pleuralis*. The snakes were found under stones or on piles of driftwood. In the stomach of one individual was a large tadpole.

Natrix sipedon pleuralis (Cope). Banded watersnake.—Found rather commonly along the smaller rocky streams of Union County, Illinois, they were also seen at Crab Orchard Lake, Williamson County, Illinois and in the swamps bordering Wolf Lake. The relations between this and the preceding subspecies are by no means clear in southern Illinois and Indiana. In places it is possible to collect typical examples of *sipedon* and *pleuralis* beneath the same rock.

Natrix sipedon confluens Blanchard. Mississippi watersnake, stump-tailed moccasin.—Specimens were collected at Big Lake National Wildlife Rufuge, Arkansas and near Cardwell, Dunklin County, Missouri. We found them only in the drier portions of the swamps where water is reduced to scattered pools clogged with driftwood and brush.

Natrix erythrogaster erythrogaster (Forster). Yellowbelly.—Specimens were collected in Union and Alexander Counties in Illinois; Dunklin County, Missouri; and Randolph County, Arkansas. DOR specimens were recorded in Butler County, Missouri and Greene County, Arkansas. We found this reptile primarily in roadside ditches and swamp pools rather than in the larger lakes and streams. One was found on a road between two cotton fields with no water nearby. This is a shy species and largely nocturnal. The Missouri and Arkansas specimens were uniformly yellow below with a tinge of pink toward the tail. Some of the Illinois specimens agreed with this description; others had a coppery-orange belly. Adults were uniformly gray-brown or blackish above; the juveniles showed indistinct transverse bands.

Natrix rhombifera rhombifera (Hallowell). Diamond-backed watersnake.—A characteristic inhabitant of cypress lakes and river swamps from southern Indiana southward, it is often seen basking on logs or overhanging bushes bordering the larger bodies of water. In the partially dry swamps, it is less common and seems to be entirely absent from clear, swiftly-flowing streams. Our largest specimen measured 48 inches and had in its stomach a 7 inch catfish. Localities where we collected *rhombifera* are: Posey County, Indiana; Alexander County, Illinois; Dunklin County, Missouri; and Mississippi and Randolph Counties, Arkansas.

Natrix cyclopion cyclopion (Duméril and Bibron). Green watersnake.—We collected two specimens along a drainage canal at Big Lake National Wildlife Refuge, Arkansas. A snake of quiet, open bodies of water, it appears very similar to *Natrix rhombifera* in habits and habitat.

Storeria dekayi wrightorum Trapido. DeKay's snake.—A single specimen was found DOR at the base of the bluffs near Wolf Lake, Illinois.

Storeria occipitomaculata occipitomaculata (Storer). Red-bellied snake.—We collected one of these snakes on a wooded ridge near Fountain Bluff,

Jackson County, Illinois. It was sunning in a tangle of wild grape vines about two feet above the ground. We have noted a similar tendency to climb in specimens of *occipitomaculata* from southern Indiana. This specimen was somewhat atypical in that the red of the underside was very faint and restricted to the median third of each ventral.

Haldea valeriae elegans (Kennicott). Smooth brownsnake.—We collected one specimen under a log in the damp hardwood forest bordering Wolf Lake, Illinois.

Thamnophis sauritus proximus (Say). Ribbon snake.—A large female was collected as it was crossing a road in the lowgrounds near Sand Ridge, Jackson County, Illinois. Sixteen young were found in its oviducts.

Thamnophis sirtalis sirtalis (Linnaeus). Garter snake.—We recorded this ordinarily common reptile only on the basis of 4 DOR specimens in Alexander County, Illinois and Posey County, Indiana. All were found near cypress lakes. In several localities, residents told us that striped snakes belonging either to this or the preceding species were very common in damp, grassy places during the early spring.

Agkistrodon piscivorus leucostoma (Troost). Cottonmouth, trap-jaw.—We found this snake only at Wolf Lake, Illinois and in the nearby swamps where it appears to be common. All we saw were in or near the water. In contrast to the harmless watersnakes which slip quickly into the water and dive at the first hint of danger, the cottonmouth swims away slowly on the surface or snaps into a defensive coil with its tail vibrating and jaws widely open. One specimen, intercepted as it swam across a patch of open water, struck at the

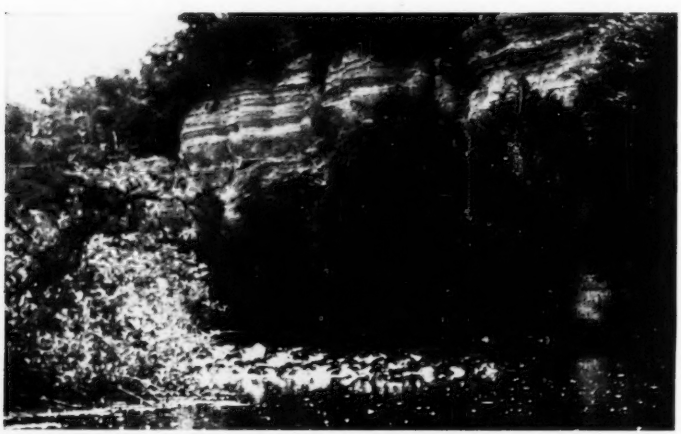

Fig. 4.—Eastern border of Wolf Lake, Union County, Illinois: crevices in the nearby cliffs provide a favorable habitat for certain snakes especially the cottonmouth, *Agkistrodon piscivorus*.

bow of our boat instead of diving to safety. We did not find the cottonmouth in the cypress lakes nor in the river swamps which are said to be the reptile's favorite habitat in the southern states.

Agkistrodon mokasen austrinus Gloyd and Conant. Copperhead.—We found a single DOR specimen in Butler County, Missouri in the transition zone between the Okark upland and the river swamps. According to other collectors and local residents, copperheads are rather common in both the Illinois and Missouri Ozarks.

Sistrurus miliarius streckeri Gloyd. Ground rattler.—One specimen was collected at Sam Baker State Park, Wayne County, Missouri. It was crossing a trail in a wooded area. Its stomach was greatly distended and contained a full-grown meadow mouse.

Crotalus horridus atricaudatus Latreille. Mountain rattler, banded rattlesnake.—An adult specimen was found basking on a rocky wooded ridge near Fountain Bluff, Jackson County, Illinois. It was captured with little show of resistance. Its stomach contained a nearly adult fox squirrel.

This reptile has 25 scale rows at midbody and in coloration is intermediate between *horridus* and *atricaudatus* although closer to the former. A resident identified this snake as a mountain rattler as distinguished locally from the diamond-back rattler of the lowgrounds. In the southeastern Missouri Ozarks, the residents refer to two large rattlesnakes, the mountain or black-tailed rattler and the diamond-back. It is likely that both these names are applied to subspecies of *C. horridus;* however it is possible that the diamond-back of southeastern Missouri may be *C. atrox.*

Amphibians

SALAMANDERS

Amphiuma means tridactylum Cuvier. Lamper. eel.—We saw a single large, mummified example in a dry portion of the river swamp in Greene County, Arkansas. According to residents, these animals frequent the smaller ditches and feed chiefly upon crayfish.

Triturus viridescens louisianensis (Wolterstoff). Louisiana newt.—Two examples of the red eft stage were collected in the swamps and damp woods near Wolf Lake, Illinois. One was beneath a rock; the other was prowling over damp leaves shortly after dark.

Ambystoma maculatum (Shaw). Spotted salamander.—One specimen was found in a pile of rotting logs on a forested ridge in Union County State Forest, Illinois.

Ambystoma opacum (Gravenhorst). Marbled salamander.—An adult female was dug from loose soil beneath a large rock at the base of a cliff at Pine Hills Recreation Area, Union County, Illinois.

Ambystoma texanum (Matthes). Small-mouthed salamander.—A sala-

mander identified as this species was found under a rotten log in the swamps near Wolf Lake, Illinois.

Plethodon glutinosus glutinosus (Green). Slimy salamander.—We saw several specimens in the hilly, forested country of southern Illinois and collected a large adult under a rotten log on a hillside 4 miles west of Jonesboro.

Eurycea longicauda longicauda (Green). Long-tailed salamander, spring lizard.—A common species in Union County, Illinois along small streams and in moist woodlands. Specimens were found under logs, rocks, and piles of damp leaves. In many cases, the hiding place was occupied by two or three animals.

Eurycea lucifuga (Rafinesque). Cave salamander, red lizard.—Several specimens were found in and around springs which open at the base of the bluffs near Wolf Lake, Illinois.

Siren intermedia nettingi Gom. Mud eel.—A small, badly decomposed specimen was found in the gullet of a *Farancia* picked up DOR near Neelyville, Butler County, Missouri.

FROGS AND TOADS

Bufo woodhousii fowleri (Hinckley). Fowler's toad.—A familiar animal in most of the localities visited. In the Ozarks, it is common along the larger stream valleys but also occurs quite high on the wooded ridges. We saw none in the dense portions of the river swamps; however they were plentiful in the cultivated fields nearby. Most specimens were seen while they were foraging after dark. Those seen during the day were usually under cover or buried in

Fig. 5.—Hardwood swamp near the northern end of Wolf Lake. Breeding area for *Hyla phaeocrypta* and other species of frogs.

loose soil. On June 12, we saw many newly-transformed young leaving temporary ponds in Union County, Illinois.

Pseudacris nigrita triseriata (Wied). Chorus frog, spring peeper.—On May 18, an occasional individual of this species could be heard calling from small, grassy ponds in Jackson and Williamson Counties, Illinois. None were collected.

Hyla cinerea cinerea (Schneider). Green tree-frog.—On June 16, we heard a large chorus of these frogs at Wolf Lake, Illinois. Calling individuals and clasping pairs were seen on projecting logs and branches or on the large leaves of the American lotus. During the day ,a few specimens were found beneath the bark of dead trees.

Hyla versicolor versicolor (LeConte). Common tree-frog.—Several specimens were collected in the swamps bordering Wolf Lake. They were heard calling during mid-June. Newly-transformed young were also seen about this time.

Hyla phaeocrypta Cope. Bird-voiced hyla.—Numerous individuals were heard in the swamps bordering Wolf Lake on the nights of June 12 and 16. The calling frogs were clinging to the trunks of trees projecting from the water, usually at heights of four to five feet above the surface. In appearance and habitat, this seems very like the preceding species; however it is smaller and its voice is a series of high-pitched, staccato notes quite different from the trilling call of *versicolor*.

Acris crepitans Baird. Cricket Frog.—This was the only hylid frog seen or heard in the river swamps and in the Missouri Ozarks. In contrast to the raucous frog choruses heard at Wolf Lake during June, the river swamps of Dunklin County, Missouri were almost silent at that time. *Acris* frequents a variety of habitats—swamps, stock ponds, roadside ditches, and the borders of large streams, lakes, and canals. Waters plentifully stocked with fish are not avoided. We collected specimens at Wolf Lake and Horseshoe Lake, Illinois; Sam Baker State Park, Missouri; and Big Lake National Wildlife Refuge, Arkansas.

Rana pipiens Schreber. Pond frog, Grass frog.—Seen in all localities visited. It is most often found in shallow ponds or ditches where there is abundant vegetation on the bank as well as in the water.

Rana clamitans Latreille. Spring frog, Greenhead.—We saw specimens at a number of localities in southern Illinois (Jackson, Union, and Alexander Counties). Observed habitats varied from springs and old wells to the borders of the larger lakes and streams.

Rana catesbeiana Shaw. Bull frog.—Large adults were seen at Wolf Lake and Horshoe Lake in southern Illinois. Tadpoles of this species were plentiful in pools of Big Creek at Sam Baker State Park, Missouri. This frog frequents large, quiet bodies of water and does not seem particularly common. It is much hunted for its meat.

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Birds in an Oak Forest during a Gypsy Moth Outbreak in South-Slovakia

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In 1947 the writer observed a disastrous outbreak of the gypsy moth (*Liparis dispar* L.) in an oak forest near the village Cankov in South-Slovakia; by June most of the trees and shrubs were leafless. Further damage resulted from the oak mildew (*Microsphaera quercina* Burr.) which followed the defoliation and brought about necrosis of the young leaves of all the native oaks.

A State Forest of about 320 hectares, 200 meters above sea-level, was studied during the first week in June. This soil is humus on a volcanic substratum with the nearest water two kilometers distant. Temperatures at noon ranged between 30° and 35° C.; the weather was dry and windless. The forest consists chiefly of Austrian oak (*Quercus cerris*) from 16 to 43 years old, with small amounts of English oak (*Quercus robur*) from 16 to 53 years old, Scotch pine (*Pinus sylvestris*) 40 to 60 years old, spruce (*Picea*) and European larch (*Larix decidua*) 40 to 55 years old. The composition of the shrub-stratum is: hedge maple (*Acer campestre*), Tatarean maple (*Acer tataricum*), elm (*Ulmus campestris*), European privet (*Ligustrum vulgare*), bloodtwig dogwood (*Cornus sanguinea*), blackthorn (*Prunus spinosa*), English hawthorn (*Crataegus oxycantha*), and European evonymus (*Evonymus europaeus*), while on the edges grow black locust (*Robinia pseudoacia*), black elderberry (*Sambucus nigra*) and goat willow (*Salix caprea*). The undergrowth consists of grasses and bird vetch (*Vicia cracca*).

Vast numbers of gypsy moth larvae were observed on the ground, on the undergrowth, bark of the trees and their branches. All of the woods and shrubs except the elderberry and common ash (*Fraxinus excelsior*) had been injured by the larvae.

In Table 1 a list is given of the birds seen on the tract in descending order of their importance as predators of the larvae, as well as four counts on three different plots.

One plot A, one hectare in size, a pure stand of Austrian oaks 40 years old on the edge of the forest, 32 individuals of 7 species were counted from 8:30 to 9:30 a.m. central European time, June 4, and 54 individuals of 15 species from 9:15 to 10:15, June 6. Plot B, of 3 hectares, was cut in 1944 and the vegetation consists of oak, blackthorn, hawthorn, mulberry and dog

I wish to thank Dr. S. Charles Kendeigh, University of Illinois, Urbana, Ill., and Mrs. Margaret M. Nice, Chicago. Ill., for helpful suggestions.

Although this article is not within the ordinary scope of this journal it is being printed because of the importance of the Gypsy moth in this country.

TABLE 1.—Birds on the area—the first 16 species listed in order of importance as predators of the larvae.

List of birds	Counts of Individuals Plot A	Plot A	Plot B	Strip
*Golden Oriole *Oriolus oriolus oriolus*	17 † ‡	16 † ‡	3	6
*Hawfinch *Coccothraustes coccothraustes*	3 † ‡	6 † ‡	7	4
*Starling *Sturnus vulgaris vulgaris*	2 ‡	4 ‡	2	3
*Chaffinch *Fringilla coelebs coelebs*	2 † ‡	4 ‡		5
*Great Tit *Parus major major*		4 † ‡		5
Hoopoe *Upupa epops*		1 † ‡		1
Cuckoo *Cuculus canorus canorus*		1 †	1	
*Great Spotted Woodpecker *Dryobates major pinetorum*				
*Red-backed Shrike *Lanius collurio collurio*		2 †	4	
*Continental Jay *Garrulus glandarius glandarius*				2
*Marsh Tit *Parus palustris stagnatilis*				
Long-tailed Tit *Aegithalos caudatus caudatus*		1		
*Hooded Crow *Corvus cornix cornix*				
*Central-European Nuthatch *Sitta europoea caesia*				3
Tree Sparrow *Passer montanus montanus*				
House Sparrow *Passer domesticus domesticus*	3 †	4 †		5
*Turtle Dove *Streptopelia turtur turtur*	4	4	2	6
*Yellow Bunting *Emberiza citrinella citrinella*	1	3		2
*Tree Pipit *Anthus trivialis trivialis*		2		
*Blackbird *Turdus merula merula*		1		2
*Blackcap *Sylvia articapilla*		1	3	
*Nightingale *Luscinia megarhyncha megarhyncha*				1
*Continental Robin *Erithacus rubecula rubecula*				1
Roller *Coracias garrulus garrulus*			1	
*Chiffchaff *Phylloscopus collybita abietinus*				

*=nesting on area; †=seen eating larvae; ‡=seen carrying larvae to young.

TABLE 1.—(*Continued.*)

List of birds	Counts of Individuals Plot A	Plot B	Strip
*Barred Warbler *Sylvia nisoria nisoria*			
*Continental Song Thrush *Turdus ericetorum philomelos*			
*Wren *Troglodytes troglodytes troglodytes*			
*Wryneck *Jynx torquilla*			

rose (*Rosa canina*) shrubs; larvae of the gypsy moth were everywhere; 23 birds of 8 species were counted from 6:00 to 7:00 a.m., June 4. The strip census was taken along a forest path one kilometer in length on June 4 from 5:00 to 5:45 a.m.; species were counted that were seen or heard as far as about 30 meters on both sides of the path; there were 46 birds of 14 species.

In the table 29 species are listed, of which 16 were seen feeding on the larvae. Four additional species were noted, but they neither nested on the area, nor were seen eating the larvae: Spotted flycatcher (*Muscicapa striata striata*), Corn Crake (*Crex crex*), Honey Buzzard (*Pernis apivorus apivorus*) and Kestrel (*Falco tinnunculus tinnunculus*). The Kestrel has been reported as eating the larvae of gypsy moths and this is also true of the Jackdaw (*Corvus monedula*) (Farsky 1933, Vertse 1944, Schwerdtfeger 1944).

It will be seen that the population was high, particularly in the one-hectare plot A; it is not known how many birds were actually breeding in this area and how many came in from outside. Unfortunately it was not possible to make counts in uninfested areas, for the whole forest was affected, nor are there any censuses of forest birds in Czechoslovakia under normal conditions. The writer believes that the population was doubled or perhaps tripled over what would have been found under ordinary conditions. The predominant species were the Golden Oriole, Hawfinch and Starling. This was also true in a gypsy moth outbreak in 1946 (Turček 1946) and in one in Hungary (Vertse 1943).

On plot B, three nests of the Red-backed Shrike were found in the defoliated blackthorn shrubs; nests of Turtle Dove and Blackcap were seen in similar situations. These nests must have been built before the branches were stripped of leaves.

A few observations were made on the quantity of gypsy moth larvae eaten by the birds: an adult Hawfinch consumed 3 larvae in one minute; stomachs of Hawfinches and Golden Orioles were filled with larvae; the stomach of a Hoopoe contained 4 larvae and other insects; stomachs of 5 Red-backed Shrikes all contained remains of gypsy moth larvae. Strangely enough, the Oriole and Hawfinch do not eat the digestive tracts of the insects, but beat

the larvae on branches until the intestines fall out and with them the larvae of the parasitic Diptera and Hymenoptera; these rejected portions were present in great numbers under the trees.

The Roller preys upon the most important predator of the gypsy moth—*Calosoma sycophanta*.

When the moths appear in early July their most important predators among the birds are the different species of Titmice, the Red-backed Shrike, Great Spotted Woodpecker, Spotted Flycatcher, Barn Swallow (*Hirundo rustica*) and Nightjar (*Caprimulgus europaeus*).

Such an infestation attracts abnormal numbers of birds into an area and enormous numbers of larvae are devoured by them, but at the same time eggs and larvae of parasitic insects are destroyed. Kendeigh (1947) studied a concentration of birds in response to an outbreak of *Archips fumiferana* in Canada; he concludes: "Probably the greatest effect of birds on the spruce budworm is to prolong the intervals between successive attacks." This may well be true in the case of gypsy moth irruptions. The actual effect of birds in controlling plagues needs further study.

SUMMARY

During a severe outbreak of the gypsy moth in South-Slovakia a concentration of birds was observed, particularly of Golden Orioles, Hawfinches, Starlings and Chaffinches, the larvae constituting the principal food of the young.

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Population Density, Life Span, and Mortality Rates of Small Mammals in the Blue-Grass Meadow and Blue-Grass Field Associations of Southern Michigan

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Introduction

The size of animal populations and fluctuations in population density have important effects on the rate of evolution of species and races. The vital statistics of populations, such as individual life expectancy and mortality rates, are closely interrelated with the survival and the evolution of animal kinds. Such information can be obtained only by long-term studies on selected sample areas.

Mammalian populations of the blue-grass meadow and blue-grass field associations in southern Michigan were first studied in the spring of 1938 with the intention of continuing this study over a period of several years. After five years of field work, this study was interrupted in 1942 by the war. The present report deals with the results of five years of trapping, at regular intervals, the small mammals of a sample area of each of these associations. The blue-grass meadow association occurs on low, poorly drained soil in southern Michigan. The blue-grass field association occurs on relatively high, well-drained soil. Descriptions of the areas studied have been previously published (Blair 1940a). All of the field work was done on the Edwin S. George Reserve of the University of Michigan, which is in Livingston County, Michigan.

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Methods

A detailed study of the small mammal populations of the blue-grass meadow and blue-grass field associations on the George Reserve was made in the spring and summer of 1938 and 1939 (Blair, 1940a, 1940b). For a measure of annual fluctuations in the density of the mammals, the same plots, with one necessary shift in location, were trapped at regular intervals in the succeeding three years. The trapping was scheduled to be done the last six days of each of the months of April, July, and October. This schedule was followed except for slight deviations enforced by unfavorable weather. Trapping in late April permits an estimate of the population size at about the onset of the breeding season. The July trapping provides a measure of the summer population, and the October trapping indicates the numbers of animals near the end of the breeding season.

A standard live-trapping technique was used (Blair, 1941b). Standard live traps (Blair, 1941a) were set in a grid pattern on the two plots being studied. During the April and October periods the traps were left set continuously, and the trapped mammals were observed, marked, and released each morning. During the July period the traps were left unset between approximately 10 A. M. and 4 P.M., as the heat of the sun would have killed most of the small mammals (principally shrews and meadow voles) caught during the day. This unsetting of the traps during the day biases the data against the forms that are active during the day. However, the traps were set during periods of greatest activity of the diurnally active species. Meadow voles reach peaks of activity at 6 to 7 A. M. and 4 to 6 P. M. (Hamilton, 1937a).

No claim is here made that this method of trapping results in the capture of every small mammal on the plot in the course of a trap period. Actually, the results of two seasons of intensive field work in 1938 and 1939 (Blair 1940a, 1940b) lead to the conclusion that most, if not all, of the small mammals old enough to leave the nest except moles (*Scalopus*) and possibly some shrews (*Sorex*) are usually recorded in the span of a trap period. The trap records give, then, at least a measure of the relative abundance of the different species of mammals, and they probably give also a fairly reliable estimate of the density of the several species. Because each small mammal was marked for future recognition, the data permit an estimate of the life span of these species in nature.

The ecological conditions of the blue-grass meadow and blue-grass field associations have been earlier described (Blair, 1940a, 1940b). The plot in blue-grass field association, comprising 3.8 acres, has been trapped at regular intervals for five years. The traps were set forty-five feet apart in lines that were sixty feet apart. The total catches of small mammals in each trap period on this plot are shown in Table I. The trapping in the blue-grass meadow asso-

ciation lacked the continuity of that in the blue-grass association. Reasons beyond my control prevented the trapping of exactly the same area over the full period. In 1938, an area comprising 2.6 acres was trapped. Before trapping was resumed the next year an artificial change had been made in the drainage of the area without consulting me. This change in drainage caused a pond, comprising about 0.9 acres to form in one end of the plot. In 1939, therefore, the plot was enlarged to include the unflooded areas of the old plot and an additional area of the same association. In 1939 the plot comprised approximately 4.1 acres. Before trapping was begun in 1940 the plot was changed slightly to prevent future flooding. From that time until the end of trapping the plot comprised 2.8 acres of blue-grass meadow. The traps on this plot were set in a grid pattern with an interval of forty-five feet between traps. The results of the trapping in 1938 and 1939 have earlier been reported (Blair, 1940b, 1940c, 1940d). The results of the trapping in 1940, 1941 and 1942 are shown in Table IV.

Populations of Small Mammals

Nine species of small mammals were taken on each trap plot. The two plots, being in different ecological associations, had markedly different densities of any given species population. The following account describes the seasonal and annual fluctuations in population density of these nine species over the five-year period, 1938 to 1942. It considers also the life span and mortality rates of certain of these species in nature as indicated by the live-trapping data.

PEROMYSCUS MANICULATUS BAIRDII

The prairie deer-mouse was generally the most abundant mammal of the blue-grass field association (Table I). This species comprised fifty per cent or more of the small mammal population on the plot in eight of the fourteen trap periods. It was the only species taken there during the April, 1940, period, and is estimated, therefore, to have comprised approximately 100 per cent of the population at that time. In only one period, April, 1941, when all populations were at a comparatively low level, were no deer-mice taken on the plot. This species was rare in the blue-grass meadow association. One was taken there in late October, 1940, and one in late October, 1941. The following account pertains, therefore, to the deer-mouse populations of the blue-grass field association.

Fluctuations in Population Density.—The deer-mice of the blue-grass field association showed marked seasonal and annual fluctuations in population size. Deer-mice were more abundant (an estimated 9.2 individuals per acre) in July, 1938, than at any subsequent time (Table II). By September of that year the population had declined to 5.8 deer-mice per acre. The years 1939 and 1940 were periods of relatively uniform, low densities of deer-mouse populations. The relatively high density, for that season, in April, 1939, evidently resulted from the carryover from the abundant numbers of the preceding year. Only three (13.6 per cent) of the mice on the plot in April, however, were resident there the preceding September. The eleven others were presumably born

after September or possibly migrated to the plot from other areas. The number of deer-mice declined slightly through 1939 and maintained a relatively constant level (approximately 1.8 to 2.4 individuals per acre) through 1940.

The density of deer-mice was lowest in April, 1941, when none was recorded on the blue-grass field plot. Following this low the population built up to a relatively high level (an estimated 6.3 mice per acre) in October of that year. There was a good carryover to the following spring (2.9 mice per acre in April, 1942), and in that year the population again built up to a high (5.5 mice per acre) in early November.

The fluctuations, from year to year, in population density of deer-mice seem to be non-cyclic in character, although the five-year period of study is too short to rule out the possibility of a cycle. In three of the years, 1938, 1941, and 1942, the deer-mice became fairly abundant (5.5 to 9.2 mice per acre), while in two of the years, 1939 and 1940, these mice maintained a low level of abundance (1.8 to 3.7 per acre). The carryover to the following spring was greater from years of comparative abundance than from years of comparative scarcity. A good spring breeding stock, however, does not necessarily predict a year of deer-mouse abundance. The spring of 1939 had the largest spring breeding stock of any year, but the population declined throughout the year. On the other hand, the plot was apparently devoid of deer-mice in the spring of 1941, but the population built up to a relatively high level by October. The breeding stock in 1941 was presumably acquired by migration from other areas. It seems probable that the fluctuations in the density of deer-mouse populations of the blue-grass field association are controlled by the interaction of many factors in this ecological community.

The density of deer-mice in any given year may have been influenced in some part by climatic conditions. Deer-mice reached their lowest seasonal maxima in the two years, 1939 and 1940, in which the rainfall for the months of April, May and June was in excess of the average for those months. Deer-mice reached their highest seasonal maxima in the years, 1938, 1941, and 1942, in which the rainfall during April, May and June was below the average for that period. The departures from the average rainfall are variable, and it is possible that the apparent correlation between amount of rainfall in the spring months and maximum annual density is due to chance. In 1939 the rainfall during April, May and June, as recorded at Milford, the nearest weather station, exceeded the average by only 0.15 inches. In 1940, the other year of low deer-mouse density, the rainfall of this period was 3.16 inches in excess of the average. In 1938, when deer-mice reached their greatest density of the five-year period, there was a deficiency of 1.65 inches. In 1941 there was a deficiency of 0.63 inches, and in 1942 there was a deficiency of 3.56 inches. There is at least a suggestion, nevertheless, that a deficiency in spring rainfall favored the increase of deer-mice, and that an excess of spring rain was unfavorable to this species. There was possibly greater flooding of the nests of this ground-nesting species in years of excess spring rainfall than in years of deficient spring rain.

The present data do not indicate whether the fluctuations on the study

plot represent local changes in numbers of deer-mice or whether they represent general regional fluctuations in numbers. There is a suggestion, at least, that the local population changes may reflect changes occurring over a much wider area. Bole (1939) found evidence that prairie deer-mice were at a peak of abundance in 1938 in the vicinity of Cleveland, Ohio. This coincides with the greatest peak of deer-mouse abundance that was found on the George Reserve during my five-year period of study. It seems possible, therefore, that the increase in numbers of deer-mice in 1938 occurred over a rather wide geographic area.

Estimated Life Span and Mortality Rates.—The length of time that a deer-mouse remained on the trap plot permits a crude estimate of the life span of an individual of this species in nature. The time an individual appeared on the plot is estimated as the mid-point of the interval between the trap period in which it was first trapped and the preceding trap period. The time that a mouse disappeared from the plot is estimated as the mid-point of the interval between the trap period in which it was last caught and the next succeeding trap period. The estimates for individual mice are rather gross estimates because of the long time interval between trap periods, but their average gives a rough indication of the length of time a mouse remained on the plot.

The average length of time a deer-mouse was on the plot, as estimated for 104 mice by the above method, was $4.88 \pm .20$ months. The shortest stay, estimated by this method, was 2.5 months, and the longest was 12.5 months. Losses from the population must have occurred in either or both of two ways. The mice either died or they moved away from the plot. No distinction can be made in the present data between losses through movement and losses through death. There is an indication, however, from the systematic trapping of extensive areas of blue-grass field association in 1938 (Blair, 1940a) that death rather than movement probably accounts for most of the population loss on the trap plot. Of twenty-six deer-mice that disappeared from a trap plot in the 1938 season, only two (7.7%) were recovered by extensive trapping of other, peripheral areas of blue-grass field association. There is no certainty, of course, that all of the mice that dispersed from the plot were recovered, but it would be expected that more than 7.7 per cent of the mice that disappeared from the plot would be retaken on the nearby areas if movement was responsible for a major part of the loss from the plot. In the records here reported, only one deer-mouse ever reappeared on the plot after being missing from a trap period. It seems likely, therefore, that most of the mice, possibly as high as 90 per cent or more of them, judging from the 1938 results, disappeared from the plot because of their death.

The apparently short life span of a deer-mouse in nature, as suggested by the average of $4.88 \pm .20$ months each mouse was on the trap plot, is very interesting by comparison with the potential life span of the deer-mouse. Deer-mice of another subspecies, *gracilis*, have been kept in captivity for as long as seven and eight years (Dice, 1933). From my own observations, the potential life span of *bairdii* is certainly in excess of three years or more. The short life expectancy of a deer-mouse in nature indicated by these data by

comparison with the comparatively long life expectancy in captivity may possibly result from one or more of several factors to be discussed later.

The mortality rates of deer-mice at the different seasons of the year are of interest here (Table III). Mortality rate, as used here, refers to all losses from the population, including the estimated ten per cent or less loss due to dispersal. Population losses can be calculated for the spring period (late April to late July), summer period (late July to late September or October) and fall-winter period (late September or October to late April). Because of the one month's difference in time of the fall trapping, it is necessary to consider the summer losses and fall-winter losses in 1938 and 1939 separately from those losses in the other years. The population losses of 86.2 per cent in the seven-month fall-winter period of 1938-39 and 1939-40 and the losses of 93.0

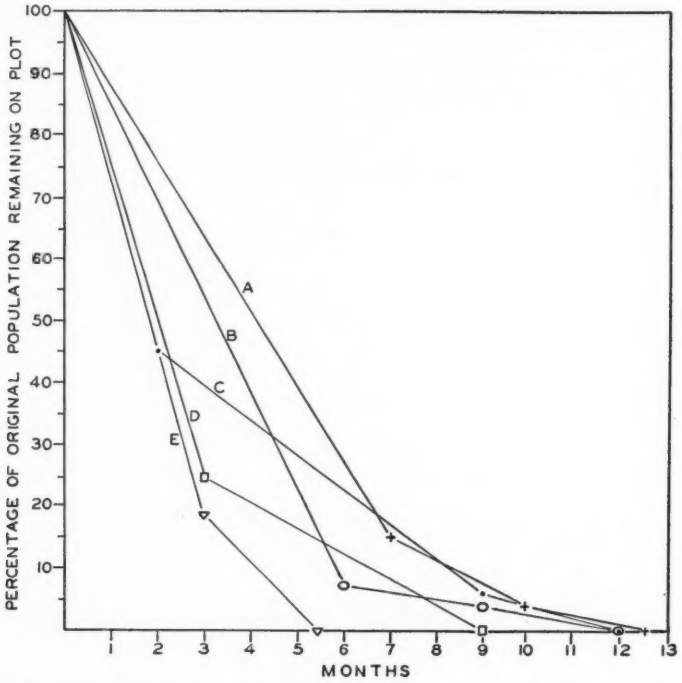

Fig. 1.—Graph showing percentages of original population of deer-mice (*Peromyscus maniculatus bairdii*) recaptured on blue-grass field association plot during one-year periods. Data for comparable seasons of different years are pooled. Ordinates are percentages of original population remaining on plot. Abcissas are elapsed months. Original populations are indicated as follows: (A) September population, 1938 and 1939; (B) October population, 1940 and 1941; (C) July population, 1938 and 1939; (D) July population, 1940 and 1941; (E) April population, 1939 to 1942, inclusive.

per cent in the six-month fall-winter period of 1940-41 and 1941-42 were only a little higher than the losses in the three-month and two-month spring and summer periods (Table III). Considering the greater length of the fall-winter period, the rate of loss during that time was probably no greater than that during the spring and summer periods. Attrition was least in the summer period, possibly because of a greater supply of food and cover. However, the differences between the different seasons are so little that they are possibly due to sampling error and to the different lengths of the respective periods.

A survival curve (Fig. 1) also permits comparison of the rate of loss from the deer-mouse population at the different seasons of the year. This curve is constructed by taking as 100 per cent for each of the respective seasons of the year the number of deer-mice recorded on the plot during the trap period immediately preceding that season. Thus the spring population would be the sum of the deer-mice recorded on the plot in late April of all the years of the study. The fall populations are summed separately for 1938 and 1939 as one group and for 1940 and 1941 as another group because of the differences in the time of the fall trapping. The summer populations are similarly divided for the same reason. The ordinates of the survival curve are taken as the percentages of the original population recorded in successive trap periods for twelve months. The curves reach or approach zero at twelve months, for no deer-mouse remained on the plot longer than that time. The survival curves for the three seasons are essentially similar. All of the seasonal populations showed their greatest loss in the first three months, after which there was a gradual flattening of the curve. As shown by these curves, survival was slightly the highest in the September populations, next highest in the July, 1938 and 1939, populations and lowest in the spring populations.

PEROMYSCUS LEUCOPUS NOVEBORACENSIS

Wood mice were recorded on the blue-grass field association plot in every trap period except one (Table I). Wood mice, in southern Michigan, however, occur principally in the oak-hickory association. The 3.8 acre plot in blue-grass field association was bounded on two sides by oak-hickory forest, and most of the wood mice recorded on the plot probably had their homes in these woods. Most of the wood mice were captured where the blue-grass field bordered the forest, and most of these mice went into the woods when released from the traps. This species rarely entered the blue-grass meadow association. One was taken there in late July, 1940. The following account, therefore, pertains to the species in the blue-grass field association.

Fluctuations in Population Density.—In the three years, 1940-1942, in which trapping was done in October or early November the number of wood mice in the blue-grass field association was greater in the fall, as indicated by these records, than in the spring or summer. This apparently indicates that the wood mouse, in this area, reaches its seasonal peak of abundance in the fall. Burt (1940), working in the oak-hickory association in 1936 and 1937, found the wood mouse most abundant in October and November.

The year to year fluctuations in the density of the wood mouse population appear to be of the same general nature as those in the prairie deer-mouse population. There is evident in the five-year record no marked cyclic alternation of years of extreme abundance with years of extreme scarcity. There is, nevertheless, a considerable amount of variation from year to year in population size. Burt (1940), whose data are based in part on live-trapping in the woodlots adjoining my plot, found wood mice to be abundant in 1936 and early 1937. My data for the succeeding years, while they are not strictly comparable with Burt's, probably do afford an index of wood mouse populations. In June, 1939, all of the wood mice living in the adjoining woodlot were taken also on the blue-grass field association plot (Blair, 1940), so the records of wood mice from the blue-grass field association probably give a fairly reliable index of the wood mouse densities of the adjacent forest. My records indicate that 1938, 1939 and 1941 were years in which wood mice were relatively scarce (Table I). The years 1940 and 1942 were times of relatively high levels of abundance.

The year to year fluctuations in numbers of wood mice in this area parallel only in part the fluctuations that Bole (1939) found in the numbers of wood mice in the vicinity of Cleveland, Ohio. Bole found a peak of abundance in 1935, but in 1936, a year of wood mouse abundance in southern Michigan according to Burt (1940), he found this species to be "positively uncommon" in the vicinity of Cleveland. Bole found wood mice abundant again in 1938, but my records show the species to have been relatively scarce in that year in southern Michigan. Lindeborg (1941) also found wood mice at "peak abundance" in central Illinois in 1938. The following year, 1939, Bole stated that the species had, "virtually vanished from Ohio's forests," and in that year I found the population to be low in my area. The partial parallelism in numbers of wood mice that Bole (1939) found in Ohio with the numbers that Burt (1940) and I found in southern Michigan suggest that some general controls of population density may have been operating in both areas. The discrepancies, however, suggest that some local controls, presumably ecological, may have been affecting the populations differently in the two areas. Bole believes that his data indicate cyclic fluctuations in abundance, with a three-year period of low populations intervening between years of peak populations. There is no evidence of such cyclic variations of wood mouse density in southern Michigan during the seven years covered by the field work of Burt (1940) and myself.

Estimated Life Span and Mortality Rates.—The average estimated time that each wood mouse was on the plot was $4.60 \pm .21$ months. As in the deer-mouse, the indicated life span in nature was much less than the potential life span of the species.

Seasonal differences in mortality rates of the wood mice were of the same general order as seasonal differences in losses from deer-mouse populations (Table III). The aggregate 1938-1939 September population of wood mice suffered 100 per cent loss in the fall-winter period, but there was a total of only eight individuals in this category. In the other periods there was even less

variation from season to season than in the case of the deer-mice. Except in the period mentioned above, the greatest loss was in the two-month period between July and late September of 1938 and 1939, when there was a loss of 88.9 per cent of the original population. The least attrition occurred in the three-month period from late July to late October of 1940, 1941, and 1942, when 74.3 per cent of the original population disappeared. The variations from season to season are, therefore, slight and probably of little significance.

The survival curves for the wood mice (Fig. 2) were much the same as the curves for the deer-mice (Fig. 1). The fall populations declined slightly less rapidly than did the spring and summer populations. It seems likely, therefore, that such possible factors as food supply had even less effect on the

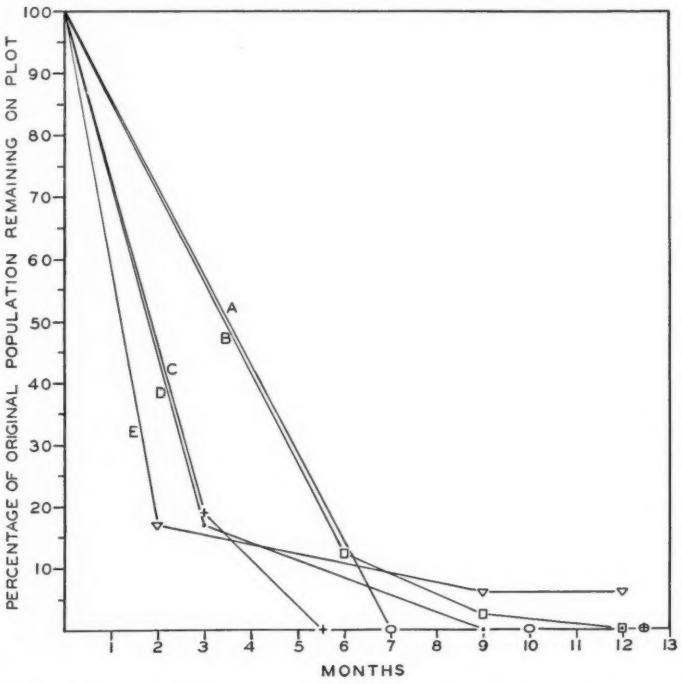

Fig. 2.—Graph showing percentages of original population of wood mice (*Peromyscus leucopus noveboracensis*) recaptured on blue-grass field association plot during one-year periods. Data for comparable seasons of different years are pooled. Ordinates are percentages of original population remaining on plot. Abcissas are elapsed months. Original populations are indicated as follows: (A) September population, 1938 and 1939; (B) October population, 1940 and 1941; (C) April population, 1939 to 1942, inclusive; (D) July population, 1940 and 1941; (E) July population, 1938 and 1939.

chance for survival of a wood mouse than on the chance for survival of a deer-mouse.

MICROTUS PENNSYLVANICUS PENNSYLVANICUS

Meadow voles were recorded in both ecological associations, but they were much more numerous in the blue-grass meadow than in the blue-grass field association. The latter association lacks the heavy ground cover that is preferred by this species, which probably accounts for the relative scarcity of this species in this community.

Fluctuations in Population Density.—The meadow vole populations of the region reached peaks of abundance in 1938 and 1942 (Table V). The 1938 peak came in mid-July, when there was an estimated density of 14.6 meadow voles per acre in the blue-grass meadow association (Blair, 1940b). The 1942 peak came in November, when there was an estimated population of 29.8 individuals per acre in this association (Table V). In the years 1939, 1940 and 1941 the population of this species was at a low ebb, for the maximum density recorded during that time in the same association was only 1.7 voles per acre. The increase in the number of meadow voles in 1942 occurred rapidly, for no voles were recorded on the blue-grass meadow plot in April. By the time of the July trapping the density of the meadow voles had reached 7.5 individuals per acre, and by the time of the November trapping it had reached 29.8 voles per acre. The decline from the peak of 1938 started immediately after the peak was reached. The density of voles in the blue-grass meadow association had dropped from the mid-July maximum of 14.6 per acre to only 5.8 per acre in late September. When trapping was resumed in April, 1939, no voles were recorded on the plot, and only three voles were taken during the entire season of trapping on the plot (Blair, 1940b). The absence of meadow voles from the trap records in several of the trap periods during the three years of meadow vole scarcity does not mean that the species had disappeared from the region. Instead, it is taken to mean that the population had reached such a low level that considerable areas of optimum meadow-vole habitat were uninhabited by the species.

Meadow voles were recorded in the blue-grass field association only when the populations of this species were at a peak in the blue-grass meadow association (Table V). In 1938 (with an estimated 10.4 voles per acre in the blue-grass meadow association) two individuals (0.5 per acre) were living in the blue-grass association plot in late September. In 1942 (with an estimated 29.8 meadow voles per acre in the blue-grass meadow association) there were eight (2.1 per acre) living in the blue-grass field association plot. The meadow voles of the blue-grass field association evidently invaded this sub-marginal habitat at time of high population pressures in the favorable habitats of the species. It is noteworthy that the meadow vole density in the blue-grass field association was roughly proportional to the density in the blue-grass meadow association. In 1942 the meadow voles of the blue-grass meadow association were almost three times as abundant as in 1938; in 1942 in the blue-grass field association they were four times as abundant as in 1938.

The peaks of abundance of the meadow vole in this region follow the four year cycle that has been reported for the species in other regions (Hamilton, 1937b). Hamilton lists previous years of high meadow-vole populations in New York state as 1919, 1923, 1927, 1931, and 1935. The 1938 high on the George Reserve was one year ahead of the expected peak, which should have come in 1939 if the population increase follows an invariable four-year cycle. Bole (1939) found an apparent three-year cycle in Ohio, for he listed 1929, 1932, 1935, and 1938 as years of peak abundance. Hamilton and Bole both list 1935 as a peak year, and the 1938 peak reported by Bole in Ohio occurred in the same year that meadow voles were abundant on the George Reserve. No trapping has been done on my plots since 1942, but conversation with several field workers indicates that meadow vole populations in southern Michigan were again at a peak in 1946.

The present evidence seems to indicate rather general concurrence of the meadow vole population densities over wide geographic areas with local interruptions of the general cycles by local ecological conditions. An adequate analysis of the factors affecting the cyclic behavior of these populations will be possible, however, only after coordinated field work in several parts of the range of the species.

Estimated Life Span and Mortality Rates.—The average time of each meadow vole on the plot, computed as for the deer-mice, shows that each vole was on the plot an average of $4.23 \pm .22$ months. These data, as do all of the data concerning vole populations, come principally from years when the species was abundant, so they possibly are representative only of years of high vole density.

The mortality rates were higher in the six and seven month fall-winter periods (late September or October to late April) than they were in the two- and three-month summer periods (late July to late September or October). No meadow vole remained on the plot through the fall-winter period (Table III). The late July populations of 1938 and 1939 had suffered a 67.9 per cent loss by the end of late September. The late July populations of 1940, 1941 and 1942 had lost 55.6 per cent of their original number by late October. The difference in fall-winter and summer survival values is evidently due to the different lengths of these periods. The fall-winter periods were six or seven months in length, while the summer periods were only two or three months in length. Survival curves for the different seasons are very similar and indicate little difference in mortality rate at the different times of the year.

BLARINA BREVICAUDA TALPOIDES

The short-tailed shrew was the second most abundant species of the bluegrass meadow during the period of study, but it was never very abundant (Table IV). There was a seasonal increase in the density of the short-tailed shrew population each year, with the maximum coming in the fall. No shrews were taken on the plot during the spring trapping in any of the three years, 1940-1942, and the maximum for the year was always recorded during the fall period. The short-tailed shrew population fluctuated very little from year to

year during the three-year period, for the fall maxima for the three years were, respectively, four, five, and five individuals on the plot. This fluctuation was less than that of the corresponding period on the blue-grass field plot, for there the fall maxima varied from nine individuals in 1940 to only three individuals in 1942.

Short-tailed shrews were taken on the plot in the blue-grass field association in four of the five years of trapping (Table I). In this area this species occurs principally in the oak-hickory association (Burt, 1940) and the blue-grass meadow association (Blair, 1940c). As in the case of the wood mice, the records of shrews from the blue-grass field association are probably indicative of the population levels in the more favorable habitats of the species. In 1939, when no short-tailed shrews were recorded from the field plot, the population of the species in the blue-grass meadow association was at a relatively low level, with a maximum for the year of only 0.7 individuals per acre (Blair, 1940c).

The data suggest that the short-tailed shrews tend to enter the blue-grass field association more in the fall than during the spring and summer. In 1940, 1941, and 1942 these shrews were recorded on the plot during the fall trapping period but at no other time. In 1938, one individual was recorded during the July period and two during the September period (Table I).

There is no evidence of cyclic variations in the numbers of short-tailed shrews on the George Reserve. Bole (1939) likewise concluded that the populations of this species are probably non-cyclic in Ohio.

A short life span is indicated for the short-tailed shrew by the records of thirty-four shrews that were released alive from the traps on the two plots. Of thirty shrews recorded alive in the fall twenty-eight were never taken again. One female that was marked in September, 1938, possibly survived as long as twenty-two months, for a female with that number was trapped in July, 1940. Another female, marked in October, 1940, possibly survived for nine or more months, as a female with the same number was trapped in July, 1940. There is no certainty that the shrews trapped the second time were the ones originally marked. These animals were marked by clipping off toes, and shrews are sometimes found with toes missing. Two of four shrews released alive in July, 1938, survived until September, but the other two were not retaken. A life span no longer than that of the wood mice, deer-mice, and meadow voles, and possibly shorter, is suggested, but the data are too few to be conclusive.

ZAPUS HUDSONIUS HUDSONIUS

The jumping mouse was rare on the George Reserve in 1938, for only one individual was taken during a season of extensive trapping (Blair, 1940d). In 1939 this species became relatively abundant and in late June reached a peak density of 5.0 individuals per acre in the blue-grass meadow association. This species became less abundant in 1940, and only eight (1.2 mice per acre allowing for the extension of home ranges beyond the limits of the plot) were recorded in July (Table IV). The jumping-mouse population density de-

creased still further in 1941, for only two (0.3 mice per acre) were recorded in July of that year. A further decrease evidently occurred in 1942, for no jumping mice were recorded that year.

The present data indicate that jumping-mouse populations in southern Michigan are subject to marked variations in density at least on local areas such as the George Reserve. The period of five years is, however, too short to determine the periodicity of any cycle that may exist. Bole (1939) failed to find evidence of a well-marked cycle in his quadrat studies near Cleveland, Ohio, but he does mention, "periodic outbreaks of this species in Ohio hayfields."

It is interesting, and possibly significant, that the peak of jumping-mouse abundance in 1939 followed by one year the 1938 peak of meadow vole abundance in the same ecological association. We do not know whether or not jumping mice increased in abundance in 1943 following the meadow vole peak of 1942, as the field work was necessarily discontinued at the end of 1942.

Only one jumping mouse was taken on the blue-grass field plot in the five-year period. This jumping mouse was trapped in July, 1939, when the species was fairly abundant (4.3 individuals per acre) in the blue-grass meadow association (Blair, 1940d). This individual apparently was not living in the blue-grass field. When released from the trap it traveled to an area of blue-grass meadow and was not again taken in the field association.

SYNAPTOMYS COOPERI COOPERI

The lemming vole fluctuates in population density in southern Michigan. Burt (1940) listed 1936 as a peak year and 1937 as a year of low numbers of this species. The population was at a relatively high level in 1938, when in late August and September five individuals were recorded on a 7.6 acre plot (Blair, 1940a). The lemming voles were living in a colony where there was a dense cover of blue-grass, so the density of the lemming population on the area occupied by the colony was greater than the small numbers might suggest. This species was scarce in 1939, and only one was found on the same area (Blair, 1940a). No lemming voles were taken in the blue-grass meadow association in 1940 or 1941. In 1942, three individuals were living on the plot in early November. It is noteworthy that in the five-year period of my study the lemming voles were most abundant in the two years, 1938 and 1942, in which meadow vole populations were at a high level. This suggests that the population levels of the two kinds of voles are possibly influenced by the same factors. Bole (1939) found evidence that lemming voles were most abundant in Ohio in 1935 and 1938. These years were ones in which meadow voles were also at a peak of abundance in that region, so Bole's observations support my observations that the density of lemming vole parallels the density of the meadow vole. Of course the peak of abundance of the lemming vole is much lower than that of the meadow vole.

Lemming voles were recorded on the blue-grass field association plot in July, 1940, and in April and November, 1942. There were no great fluctuations in the abundance there of this relatively scarce species.

OTHER SPECIES

Three species of small mammals were scarce in both ecological associations. One house-mouse (*Mus musculus*) was taken on each plot during the five-year period. Long-tailed shrews (*Sorex cinereus*) were taken on the blue-grass meadow plot once in the fall of 1941, once in the spring of 1942 and once in the fall of 1942. One was taken on the blue-grass meadow plot in the fall of 1942. It is possible that these shrews represented only a fraction of the *Sorex* population, for these animals are rather small to trip the mechanism of the traps that were used. The few *Sorex* that were caught in 1941 and 1942 possibly indicate an increase in the numbers of *Sorex* at those times. None of these animals was taken in the first three seasons of trapping the two associations.

Thirteen-lined spermophiles (*Citellus tridecemlineatus tridecemlineatus*) were recorded in the blue-grass field association in 1940, 1941 and 1942, and in the blue-grass meadow association in 1941 and 1942. This species is relatively scarce in the area. There is no indication in these data of any marked cyclic variations in population size.

Fluctuations in Density of Small Mammal Populations

The records here presented of a five-year field study show that seasonal and annual fluctuations in population density may differ greatly in different species of small mammals occurring in the same region or in the some ecological association. The meadow vole, lemming vole, and jumping mouse exhibit marked year to year changes in abundance. Of these, the meadow vole at least follows a regular cycle of abundance, with peak densities coming every three or four years (Hamilton, 1937b: Bole, 1939). Two other species, the wood mouse and prairie deer-mouse vary considerably in numbers from year to year, but the maxima and minima of population density are less extreme than in the meadow vole. The short-tailed shrew shows but little annual variation in population density in this region, and the densities remain at a low, fairly uniform level. The thirteen-lined spermophile and house-mouse remain minor components of the blue-grass meadow and blue-grass field associations from year to year. Shrews of the genus *Sorex* appear to be rare here.

The differences between the several species regarding average levels of population density and regarding seasonal and annual fluctuations in density presumably are basically genetic. They are genetic in the sense that the genetically determined morphology, physiology and possibly psychology of each species must determine, in large part, the habits of the species and determine its place in the complex ecological association of plants, animals and physical environment. The change in density of any given species undoubtedly reflects changes in the complex of factors that interact in the association and that operate directly or indirectly to limit population densities. The interplay of factors that limit the density of the several species of mammals in a community such as the blue-grass meadow or blue-grass field association is undoubtedly a complicated process. The present data do not suffice for an analysis of this

complex system of interacting factors, but they do provide a clue to the role of some of the factors.

Closely related species do not seem to show parallel trends in population density unless there is also similarity in the ecological niche occupied. Deer-mice and wood mice, which are closely related species but which have different ecological preferences, show little resemblance in population densities. Both species were relatively scarce in 1939. Wood mice were much more abundant than deer-mice in 1940 and 1942. Deer-mice, on the other hand, were more than four times as abundant as wood mice in 1938 and nearly twice as abundant as wood mice in 1941. It is evident from these data that the factors limiting population densities were different for the two species or they affected the two species differently.

Certain of the species having fairly similar habits show some parallelism in population densities regardless of whether they are closely related or not. The closely related meadow vole and lemming vole and the more distantly related deer-mouse occupy subterranean nests and are closely associated with the blue-grass (*Poa pratensis*). All three species showed relatively high population densities in 1938 and 1942. All three had low densities in 1939 and 1940. The meadow vole and lemming vole were also scarce in 1941, but the deer-mouse was relatively abundant in that year. It seems probable, therefore, that the same factors were operating to limit in a general way the densities of these three species.

Interspecific competition seems likely to be an unimportant factor limiting the density of these species. Since the deer-mouse occurs principally in the blue-grass association and the meadow vole occurs principally in the blue-grass meadow association, there is little opportunity for competition between the two species. Some competition possibly occurs when meadow voles, during periods of high density, spread into the areas occupied by deer-mice. Such competition could possibly account for the fact that deer-mice were less abundant on the upland plot in the fall of 1942, when eight meadow voles were living there, than in the fall of 1941, when no meadow voles were recorded there. Lemming voles, which occur principally in moist swales on the dry upland, possibly encounter some competition from invading meadow voles and from deer-mice. Where the lemming voles occur with meadow voles in the blue-grass meadow association, there is possibly competition between the two species to the disadvantage of the lemming voles. In the fall of 1942, there were only three lemming voles on the blue-grass meadow plot by comparison with 139 meadow voles.

The jumping mouse failed conspicuously to parallel the other ground inhabiting mammals of the blue-grass meadow association in population density. Jumping mice were comparatively common (an estimated 5.0 individuals per acre) in 1939 and were scarce in the four other years. There is a suggestion at least of a possibly competitive relationship between meadow-vole and jumping-mouse population densities. The jumping mouse and meadow vole both occur principally in the blue-grass meadow association. The greatest density of jumping mice came in 1939, a year in which meadow voles reached

their greatest scarcity of the five-year period (Table V). Conversely, the two years of greatest scarcity of jumping mice were 1938 and 1942, when the meadow vole reached peaks of population density. There seems to be, from the consistency of this relationship over the five-year period, the interesting possibility of some adjustment between the population densities of the two species. Present data are insufficient for more than a suggestion of such a relationship.

Life Span and Mortality Rates of Small Mammals

The life span of small mammals in nature has received some attention from population workers in recent years, but the estimates of the life duration of wild small mammals have been for the most part vague and crude. Baker and Ranson (1933) state that most mature meadow voles (*Microtus agrestis*) die in the fall or early winter and thus seldom survive more than one year. Hamilton (1937c) states in regard to *Microtus pennsylvanicus*, "The adult mice ostensibly die in the late winter, when less than a year old. In the case of those born late in the fall, the mice perhaps over-winter, but these individuals die when slightly more than one year old." These workers used weight as a criterion of age in samples of populations. Burt (1940) working with the wood mouse (*Peromyscus leucopus noveboracensis*) followed marked populations for more than two years and concluded that, "Most mice of this species probably are eliminated by predators or die from some other cause before they reach the age of one year." Blair (1940a) followed marked populations of deer-mice (*Peromyscus maniculatus bairdii*) through two seasons and concluded that there is an almost complete annual turnover in the population of this species. Blair (1941c) working with the woodland deer-mouse (*Peromyscus maniculatus gracilis*) estimated that approximately 6.1 per cent of an August population represented a carryover from the previous year. Pearson (1945) followed marked populations of wood mice (*Peromyscus leucopus noveboracensis*) and of short-tailed shrews (*Blarina brevicauda*). He found that six per cent of sixty-seven shrews survived from one summer to the next and that four per cent of sixty-nine wood mice survived a like time. All of these workers agree that in general the life span of small mammals of several different species is usually less than one year. The present data give, for three species of small mammals, a more specific estimate of the life span than has been heretofore available.

Prairie deer-mice (*Peromyscus maniculatus bairdii*) lived on my plots an average of $4.88 \pm .20$ months. Wood mice (*Peromyscus leucopus noveboracensis*) lived there an average of $4.64 \pm .21$ months. Meadow voles lived there an average of $4.23 \pm .22$ months. Dispersal movements probably account for some of the population loss, but, for reasons already stated this is believed to account for less than ten per cent of the loss. The data for short-tailed shrews (*Blarina brevicauda talpoides*) are too few to be conclusive, but the indication is that these animals live no longer in nature than these other species. It appears evident from these data that an individual of these species rarely lives long enough in nature to die of old age. Mice of the genus *Peromyscus* ordinarily

live to an age in excess of three years in captivity. Dice (1933) reported some individuals of this genus living to ages of seven and eight years. Pearson (1945) kept short-tailed shrews in captivity to ages of as much as thirty-three months. Bailey (1924) kept meadow voles in captivity in excess of one year. In all of these species, then, the actual life span in nature as indicated by my data, is much shorter than the potential life span, and it is probably rare indeed that an individual lives out its potential life span in nature.

Not all recent workers are in agreement that the actual life span of a small mammal is generally less than its potential life span. Hamilton (1942) states that, "in most small mammal populations, there is a distinct turnover of the population each winter, the adults of many species dying of senility or some other cause after they have passed through a single breeding season. The majority of shrews, deer mice, jumping mice, red-backed mice and species of similar size die when they have attained an age of 15 to 22 months." This same worker (Hamilton, 1940) writes in regard to the life span of the smoky shrew (*Sorex fumeus fumeus*), "The life span of the smoky shrew is short, for all the adults die of old age when they are 14 to 17 months old."

There is no evidence that a single individual of any species on my plots lived out more than a fraction of its potential life span, and, as already stated, the average actual life span in nature for three species is estimated from my data to be only four to five months. The observations of Burt (1940) on marked populations of the wood mouse (*Peromyscus leucopus*) in nature and of Pearson (1945) on marked populations of wood mice and of short-tailed shrews (*Blarina brevicauda*) likewise indicate that old age is seldom a cause of death in wild populations of small mammals.

The characteristic change in population density of small mammals from peak densities in the fall of the year to low densities in the spring has apparently led Hamilton (1937c, 1940, 1942) to the belief that death from old age as postulated by him occurs mostly in the fall or winter.

My data, however, reveal no notably greater mortality in winter than at other seasons. Survival curves for the wood mouse (Fig. 2) deer-mouse (Fig. 1) and meadow vole are remarkably similar regardless of whether the curve is constructed for an initial spring, summer, or fall population. The individual small mammal's chances of surviving are, therefore, roughly equal at all seasons of the year. The chance of survival is just as great in winter as in any other period of the year. Winter reduction in population densities can be explained without postulating a sudden die-off of a majority of the population from old age. We have presented evidence to show that the chances of survival, or conversely of mortality, of any given small mammals of the species here considered are roughly the same at all seasons of the year. Population density increases from the onset of the spring breeding season to the cessation of breeding in the fall because attrition is more than balanced by the birth of new individuals. The potential rate of increase of these species is such that the population density tends to increase during the breeding season in spite of heavy attrition. Blair (1940a) estimates that if all offspring survived (a hypothetical situation that probably never obtains) each original pair

of prairie deer-mice would have 48 descendants at the end of a breeding season. Burt (1940) estimates that, under the same hypothetical conditions of complete survival, an initial pair of wood mice would have 32 descendants at the end of one breeding season. Bailey (1924) estimates, not without some exaggeration, that, with complete survival, one initial pair of meadow voles would be represented by one million descendants at the end of a year. After breeding ceases in the fall, and until the onset of reproduction in the spring, population density decreases because there is no production of new individuals to offset the continued, heavy attrition. The winter decrease in numbers of small mammals, therefore, results principally from the lack of breeding during the winter months. These populations show, then, a pattern of survival in which the chance of survival of a given individual varies but little from season to season. Increase of population density during the breeding season does not seem to increase the chance of survival of any given individual. This effect is explained if, as McAtee (1933) believes, "predation and parasitism tend to be proportional to population, however rising and falling progressively with the increase or decrease in the number of available food organisms." In other words, as population density increases the overall attrition on the population would increase, but it would not necessarily follow that the chance of survival of a given individual would be increased or decreased. Errington (1946) seems to hold the same view.

Burt (1940) likewise believes that changes in population density of the wood mouse are related to changes in breeding activity. He states, "The population curve throughout the year reflects the breeding activities of the animals. . . . With the breeding season ended, the curve descends gradually until the lowest point of the year is again reached the following May." Pearson (1945) also believes that the population curve of the short-tailed shrew is related to breeding activity, and he demonstrates this graphically (*idem:* Fig. 2).

The remarkably short life span of the small mammals indicated by the data here reported could have resulted from several ecological factors, but some of the factors were probably more important than others. Disease, parasitism, starvation, emergencies due to weather, accidents and predation all could possibly contribute to the short life span of these small mammals. Disease organisms have been described from many populations of small mammals (Elton, 1942), but the species here considered have been little studied for disease. Hamilton (1937b) believes that an unidentified disease was responsible for reducing meadow vole populations at a time of peak density. Burt (1940) in more than two years of field work found three dead wood mice, but he found no evidence of disease. There is no evidence in my data that disease played any important part in shortening the life of the small mammals, but it is possible that disease did play a part in reducing peak densities of meadow voles. No animal showed symptoms of disease, nor was a dead animal of any species found on the plots. It seems unlikely, therefore, that disease played any great part in reducing the life expectancy of the small mammals. Few ectoparasites were observed on these animals. Wood-mice were parasitized by bot-fly larvae in late summer, but these parasites seemingly had

no critical effect on survival. Burt (1940) found scab mites and bot-fly larvae parasites on wood mice, but he reports that all individuals under observation recovered from these infections.

Shortage of food supply and attendant starvation cannot account for the shortened life spans, for attrition rates were little different at times of possible scarcity (i. e., winter) than they were at times of presumed plenty. The slightly higher rate of survival of meadow voles in summer (Table III) was possibly related to food supply. Wood mice and deer-mice, on the other hand, had slightly higher rates of survival in winter than in summer (Figs. 1 and 2). Hamilton (1942) likewise believes starvation to be of little importance for he writes, "all specimens taken during the winter months had well-filled stomachs and intestines and have appeared in first rate physical condition." Errington (1946) holds to the same view, for he writes, "the population levels maintained by most species that have been carefully studied seldom appear to be linked very closely with gross differences in food and cover, once minimal requirements have been met."

Emergencies of weather, such as heavy rainfall or sudden thaws, possibly account for the deaths of a few small mammals, but these effects are difficult of evaluation. There is no direct evidence in my data of such factors affecting the life of any small mammal on the study areas, but there is a suggestion that excess spring rainfall may have had some adverse effect on the deer-mouse populations in 1939 and 1940.

Predation is the most logical explanation of the short life span of a small mammal, at least in the area of my study. Known predators on small mammals are plentiful in this region. Red foxes (*Vulpes fulva*) and weasels (*Mustela frenata*) occur in the blue-grass meadow and blue-grass field associations. Short-tailed shrews, which occur in these associations, prey on small rodents. Five species of owls, the American barn owl (*Tyto alba pratincola*), northern barred owl (*Strix varia varia*), long-eared owl (*Asio wilsonianus*), eastern screech owl (*Otus asio naevius*) and the great horned owl (*Bubo virginianus virginianus*) are permanent residents in the region, and the short-eared owl (*Asio flammeus flammeus*) is a summer resident (Boardman and Barto, 1937). Blue racers (*Coluber constrictor flaviventris*) are active in both the blue-grass field and blue-grass meadow associations in summer.

Direct evidence of predation is necessarily limited. Remains of most of the species of small mammals recorded on the plots were found in pellets of the screech owl and long-eared owl that were picked up on and in the vicinity of the George Reserve. A weasel (*Mustela frenata*) forced its way into a trap and devoured a jumping mouse. Murie (1936) reported remains of all of the species of small mammals on my plots in fox kills on the George Reserve. A study of predator—prey relationships in a banded population of small mammals would be extremely valuable for a better understanding of the exact influence of predation on populations. Even without such a conclusive study, the evidence is strong that predation is probably the most important cause of the comparatively short life span of a small mammal in nature, at least in the area studied.

Summary

Fluctuations in populations of small mammals were studied in areas of the blue-grass field and blue-grass meadow association of the Edwin S. George Reserve, Livingston County, Michigan, from 1938 through 1942. Several plots were trapped intensively in both associations during the field seasons of 1938 and 1939. The resident small mammals were marked and released alive at the point of capture. During the following three years, one of the established plots in the blue-grass field association and a plot in the blue-grass meadow association were trapped approximately the last six days of April, July and October.

The meadow vole (*Microtus pennsylvanicus pennsylvanicus*), lemming vole (*Synaptomys cooperi cooperi*) and jumping mouse (*Zapus hudsonius hudsonius*) exhibit marked annual variations in abundance in this area. Meadow vole populations were high in 1938 (with an estimated peak of 10.4 voles per acre) and 1942 (with an estimated maximum of 29.8 voles per acre), and lemming vole populations were higher in those years than in the other years of study. Jumping-mouse populations were high in 1939 (with an estimated maximum of 5.0 mice per acre) and low in the other years. The density of prairie deer-mouse (*Peromyscus maniculatus bairdii*) and wood mouse (*Peromyscus leucopus noveboracensis*) populations varied considerably from year to year but showed no changes in density approaching the magnitude of the changes in density of voles and jumping mice. Deer-mice were relatively abundant in the blue-grass field association in 1938 (9.2 per acre), 1941 (6.3 per acre) and 1942 (5.5 per acre), and they were relatively scarce in 1939 (3.9 per acre) and 1940 (2.4 per acre). Wood mouse populations were high in the blue-grass field association in 1940 and 1942, and they were relatively low in 1938, 1939 and 1941. Populations of the short-tailed shrew (*Blarina brevicauda carolinensis*) tended to remain at a low, relatively uniform level. The thirteen-lined spermophile (*Citellus tridecemlineatus tridecemlineatus*) house-mouse (*Mus musculus*) and a long-tailed shrew (*Sorex cinereus*) were minor components of the mammal population.

Closely related species failed to show parallel trends in population density unless there was also similarity in ecological habits. Wood mice were much more abundant than closely related deer-mice in 1940 and 1942. Deer-mice, on the other hand, were much more abundant than wood mice in 1938 and 1941.

Species with generally similar habits showed some concurrent trends in population density regardless of degree of phylogenetic relationship. Meadow voles, lemming voles and deer-mice were relatively abundant in 1938 and 1942, and these same species had low population densities in 1939 and 1940. The jumping mouse was a notable exception, for this species was abundant in 1939, a year of scarcity of these other species, and it was rare in other years.

The average actual life span in nature of several species was estimated from the trap records. The prairie deer-mouse is estimated to have remained on my plots an average of $4.88 \pm .20$ months. The wood mouse is estimated to have lived on the plots an average of $4.64 \pm .21$ months. The meadow vole is estimated to have lived on the plots an average of $4.23 \pm .22$ months. Ten

per cent or less of the loss from the plots is believed to be due to dispersal. Therefore, the short average time an animal lived on the plots is taken to be indicative of a short actual life span in nature for these species. The actual life span of the short-tailed shrew is indicated by the available data to be of the same order as that of the other species. The indicated actual life span in nature for all of these species is much shorter than the potential life span as determined from captive animals.

The short life span of a small mammal in nature is mostly attributable to predation. Other factors, such as disease, parasitism, starvation, weather emergencies and accidents, could possibly aid in shortening life expectancy, but their effects seem to be relatively minor by comparison with the effects of predation. Death from old age is a rare phenomenon in these populations.

The chance of survival of an individual small mammal of the species studied in this region appears to be approximately the same at all seasons of the year. Survival curves for the wood mouse, deer-mouse and meadow vole are remarkably similar regardless of whether the curve is constructed for an initial spring, summer or fall population. There is no evidence of an appreciably greater mortality rate in winter, as suggested by some authors to explain winter reduction in population density, than at other seasons. With a generally uniform, high rate of attrition throughout the year, as demonstrated in these populations, winter reduction in population density is explained principally as the result of failure to breed during the winter months.

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TABLE I.—Numbers of individual small mammals captured during a five-year live-trapping census on 3.8 acres of blue-grass field association on the Edwin S. George Reserve, Livingston County, Michigan.

	Peromyscus maniculatus bairdii	*Peromyscus leucopus noveboracensis*	*Microtus p. pennsylvanicus*	*Blarina brevicauda talpoides*	*Citellus t. tridecemlineatus*	*Synaptomys c. cooperi*	*Sorex cinereus*	*Zapus h. hudsonius*	*Mus musculus*
July 25-31, 1938	35	8		1					
Sept. 21-27, 1938	22	5	2	2					
April 23-26, 1939	14	3							
July 23-29, 1939	11	10						1	
Sept. 17-23, 1939	7	2							
April 27-May 2, 1940	7								
July 26-31, 1940	8	18					1		1
Oct. 26-31, 1940	9	30		9	1				
April 25-30, 1941		3			4				
July 26-31, 1941	4	5							
Oct. 24-28, 1941	24	13		5					
April 25-30, 1942	11	4			3	4			
July 24-29, 1942	14	12	1		8				
Nov. 10-13, 1942	21	47	8	3		2	1		

TABLE II.—Number of individual deer-mice (*Peromyscus maniculatus bairdii*) trapped on 3.8 acres of blue-grass field association, carryover from one period to the next, and estimated populations per acre. Edwin S. George Reserve, Livingston County, Michigan.

TRAPPING PERIOD	Number remaining from previous period (all adults)	Previously unmarked adults	Immature mice (previously unmarked)	Total mice recorded	Estimated population per acre
July 25-31, 1938	0	29	6	35	9.2
Sept. 21-27, 1938	12	7	3	22	5.8
April 23-26, 1939	3	11	0	14	3.7
July 23-29, 1939	2	10	3	15	3.9
Sept. 17-23, 1939	5	1	1	7	1.8
April 27-May 2, 1940	1	6	0	7	1.8
July 26-31, 1940	2	3	3	8	2.1
Oct. 26-31, 1940	0	8	1	9	2.4
April 25-30, 1941	0	0	0	0	0.0
July 26-31, 1941	0	3	1	4	1.1
Oct. 24-28, 1941	3	16	5	24	6.3
April 25-30, 1942	2	9	0	11	2.9
July 24-29, 1942	1	12	1	14	3.7
Nov. 10-13, 1942	3	12	6	21	5.5

TABLE III.—Seasonal losses from deer-mouse (*Peromyscus maniculatus bairdii*), wood mouse (*Peromyscus leucopus noveboracensis*) and meadow vole (*Microtus pennsylvanicus pennsylvanicus*) populations over a five-year period on the Edwin S. George Reserve, Livingston County, Michigan.

	Periods	Number recorded at beginning of period	Number remaining at end of period	Percentage lost in period
Deer-mouse	Late April to late July 1939-1942	32	5	84.4
	Late July to late September, 1938, 1939	50	18	64.0
	Late July to late October, 1940-1942	26	6	77.0
	Late September to late April, 1938, 1939	29	4	86.2
	Late October to late April, 1940, 1941	33	2	93.9
Wood Mouse	Late April to late July, 1939-1942	11	2	81.8
	Late July to late September, 1938, 1939	18	2	88.9
	Late July to late October, 1940-1942	35	9	74.3
	Late September to late April, 1938, 1939	8	0	100.0
	Late October to late April, 1940, 1941	43	5	88.4
Meadow Vole	Late April to late July, 1939-1942	1	0	100.0
	Late July to late September, 1938, 1939	28	9	67.9
	Late July to late October, 1940-1942	36	16	55.6
	Late September to late April, 1938, 1939	15	0	100.0
	Late October to late April, 1940, 1941	16	0	100.0

TABLE IV.—Numbers of individual small mammals captured during a three-year live-trapping census on 2.8 acres of blue-grass meadow association, Edwin S. George Reserve, Livingston County, Michigan.

	Peromyscus maniculatus bairdii	*Peromyscus leucopus noveboracensis*	*Microtus p. pennsylvanicus*	*Blarina brevicauda talpoides*	*Zapus h. hudsonius*	*Synaptomys c. cooperi*	*Mus musculus*	*Citellus t. tridecemlineatus*	*Sorex cinereus*
April 27-May 2, 1940									
July 26-31, 1940		1	1		8				
Oct. 26-31, 1940	1		8	4					
April 25-30, 1941			1					1	
July 26-31, 1941				3	2		1	1	
Oct. 24-28, 1941	1		8	5					1
April 25-30, 1942									1
July 24-29, 1942			35	4				2	
Nov. 10-13, 1942			139	5		3			1

TABLE V.—Numbers of individual meadow voles (*Microtus pennsylvanicus pennsylvanicus*) trapped on sample plots, and estimated populations of meadow voles per acre of the blue-grass meadow and blue-grass field association on the Edwin S. George Reserve, Livingston County, Michigan.

Trapping Period	Blue-Grass meadow Plots		Blue-grass field Plot	
	Number trapped	Estimated population per acre	Number trapped	Estimated population per acre
May 21-23, 1938	3	1.1	Not trapped	
July 25-31, 1938	27	10.4		0.0
Sept. 21-27, 1938	15	5.8	2	0.5
April 23-26, 1939		0.0		0.0
July 23-29, 1939	1	0.2		0.0
Sept. 17-23, 1939		0.0		0.0
April 27-May 2, 1940		0.0		0.0
July 26-31, 1940	1	0.2		0.0
Oct. 26-31, 1940	8	1.7		0.0
April 25-30, 1941	1	0.2		0.0
July 26-31, 1941		0.0		0.0
Oct. 24-28, 1941	8	1.7		0.0
April 25-30, 1942		0.0		0.0
July 24-29, 1942	35	7.5	1	0.3
Nov. 10-13, 1942	139	29.8	8	2.1

Variation in the Deer-Mouse (Peromyscus maniculatus) Along the Lower Columbia River*

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Introduction

Studies of variation in populations of the deer-mouse (*Peromyscus maniculatus*) have been made by Summer, Dice, and others. Dice, especially, has presented comparisons of populations of many races throughout most of the range of the species. The picture of variation in this species is known more completely than that in any other species of wild mammal. Therefore it seemed pertinent to analyze the variation of the population in an area which has not been covered by Dice. The study is based on specimens in the Museum of Vertebrate Zoology of the University of California.

There is a marked difference between Dice's technique and mine; since my work is based on ordinary study skins prepared in the field by various collectors, whereas that of Dice is based on flat skins of captive-bred animals. Dice's material has the advantages of larger samples, of skin measurements taken by one person, and of being composed of mice of the same age. My study has the advantage of dealing with samples of a natural population.

The area involved is the Columbia River Basin west of the Snake River, although a few specimens from Walla Walla and Columbia counties, Washington, and Wallowa County, Oregon, were included. Otherwise, only specimens from those counties immediately adjacent to the Columbia River in Washington and Oregon were used. The reason for this was that most of our specimens were collected along with many other species of mammals for the purpose of studying the effect of the Columbia River on distribution. Comments concerning the effect of the river on the distribution of *Peromyscus maniculatus* will follow in the discussion.

The Columbia River Basin includes diverse habitats. Extremes are found at opposite ends of the river. At the mouth of the river there are heavy forests of Douglas fir and Sitka spruce with dense undergrowth. At the level of the Cascades yellow pine predominates and the undergrowth is much thinner. In the more arid regions east of the Cascades, bunch grass and sagebrush are present. Coniferous forests are again present farther east in the Blue Mountains.

According to the present classification, five races of *Peromyscus manicula-*

* Contribution from the Museum of Vertebrate Zoology and the Department of Zoology, University of California, Berkeley.

tus enter this area (Osgood, 1909; Bailey, 1936; Dice, 1939). On the north side of the Columbia River *P. m. oreas* occupies the coastal area of western Washington. East of this in Cowlitz and Clark counties, *P. m. austerus* is thought to occur with *oreas* but not to interbreed with it. South of the river in western Oregon, *P. m. rubidus* occupies a habitat closely similar to that occupied by *oreas*. East of the Cascades *P. m. gambelii* occurs on both sides of the river throughout the grassland-sagebrush association and into the Lower Transition Zone. It intergrades with *rubidus* south of the Columbia River (Osgood, 1909: 66) and with *oreas* north of the river. Osgood does not mention intergradation with *oreas* except at the northern end of the range of *gambelii* where he says that "the gradation from *gambeli(i)* via *artemisiae* to *oreas* seems to be complete, although at certain localities representatives of each may be found apparently preserving distinctness" (Osgood, 1909: 59). The slightly larger, darker specimens in the Blue Mountains which intergrade with *gambelii* are included in the northern race, *P. m. artemisiae* by both Dice (1939) and Osgood (1909: 60).

Acknowledgments.—Most of the specimens used in the study were collected by members of field parties of the Museum of Vertebrate Zoology. Field notes of Ward C. Russell, D. F. Hoffmeister, John Davis, J. E. Chattin, L. Tevis and Albert Wolfson supplied information concerning the habitats in which the animals were caught. This study was started under the direction of Dr. E. R. Hall and was continued under Dr. S. B. Benson and Dr. A. H. Miller. Helpful instructions for operating the Durham Reflectometer were willingly given by John E. Gullberg. I am also grateful to Stanley G. Jewett, Alex Walker and Walter Dalquest for information obtained in personal communications.

Procedure.—Two hundred and eighty-one adult specimens in the collection of the Museum of Vertebrate Zoology form the basis of this study. Most of these specimens were collected in the months of July and August of 1939, 1940, and 1941. Some were taken in May and June of these years. The Astoria population was collected in January, 1927, by Alex Walker. The Oak Slough Bank population was collected in December, 1915, by S. G. Jewett. Most of the specimens are therefore roughly comparable as regards seasonal pelage.

MEASUREMENTS

All the measurements employed by Dice (1932, 1939), with the exception of femur length, were used. In addition, other measurements were taken to test other possible variations. These are:

Condylo-basilar: from the posterior margin of the occipital condyle to the base of the incisor at its posterior margin.

Diastema: from the posterior margin of the alveolus of the incisor to the anterior margin of the alveolus of the first cheek tooth.

In the early stages of the study measurements were taken of the frontals, parietals, interorbital breadth, and tooth row, but these proved to be too highly variable in a given population to establish significant differences and were discontinued.

Two measurements of skull length, condylo-basilar and condyle-premaxillae,

were taken. This near duplication was performed in order to test which one would show less individual variation and therefore be the more satisfactory measurement. Although there is no significant difference in the means of the coefficients of variation of the two measurements in the seventeen samples, the distribution of the coefficients of variation shows that condylo-basilar length is more variable than condyle-premaxillae length. Hence, the latter seems the preferable one to use.

All skull measurements were taken by the author. The skin measurements were taken from the specimen labels. These were made in the field by trained collectors but personal error is doubtless present. However, the methods of these collectors are standardized sufficiently to keep this low.

Color measurements were made on the posterior part of the dorsal stripe by using the Durham Reflectometer (Durham, 1941). Separate readings were made of the current from the photronic cell in millimeters of galvanometer deflections using red, green, and blue filters which were especially designed to include as complete a spectral range as was practicable. The readings from the skin were then transformed to percentages of the reading for the standard (magnesium carbonate block) with each filter. By constant check on the readings for the standard, the percentages obtained could be accurately compared.

The method proposed by Dice and Leraas (1936) was used to test significant differences between samples. Whenever this graphic representation indicated only a slight significant difference, the statistical test of $d/\sigma_d = 3$ was applied. Hubbs and Perlmutter's (1942) support of the formula $\sigma d = \sqrt{\sigma^2 m_1 + \sigma^2 m_2}$ was accepted and this formula was used in preference to the longer one advocated by Simpson and Roe (1939: 193).

SEXUAL VARIATION

In order to test differences between the sexes the measurements for each sex were indicated in a different color on graphs showing the distribution of each measurement in each sample. When inspection of the graph indicated an average difference between the sexes in a particular measurement, it was tested statistically. Results showed that only one of the differences was statistically significant. In the Fort Clatsop population the females average 5.17 mm. longer in body length than the males. The difference between the means is about 4 times the standard error of the difference, and is therefore statistically significant. A similar situation is present in the Wasco and Sherman counties sample. Summer (1918) found that the males of *Peromyscus* tend to have larger feet than females. Dice (1932) found that in *Peromyscus maniculatus bairdii* the males tend to exceed the females in foot length, weight, length of tail, and bullar width. Dice (1933) found that the males of *Peromyscus maniculatus rufinus* had larger feet and were heavier than the females. The samples used by Dice were large enough to determine that there are sexual differences in size in the genus *Peromyscus*, but for the most part, the differences are slight and appear not to be significant. This is in agreement with my

results and I have followed Dice in the practice of including males and females in the same population sample.

AGE VARIATION

Age variation did not appear to be significant in specimens that no longer showed juvenal pelage and which show at least a slight wearing on the last molar. Only those of this type or older were used in the samples. The younger of the *Peromyscus* falling within this age limit seem for the most part to have attained full adult proportions.

SAMPLE VARIATION

The samples are not as homogeneous as is ideally desirable because of the range of age included and because it was necessary in a few cases to combine several small samples of close geographic proximity. The effect of this is reflected in the coefficients of variation (Tables I-III) which are larger than those recorded by Dice for more highly selected and standardized samples. The following table compares the coefficients of variation of the Klickitat-Benton sample (column 5), one of the more scattered samples, with figures taken from Dice (1933: 27, Table XI) for *Peromyscus maniculatus rufinus* (column 1-4) from Cloudcroft, New Mexico. My figures compare favorably with his for the wild-killed mice but indicate greater variability than his cage-reared animals.

	Wild-killed	Field-caught	1st year age class	2nd year age class	Klickitat-Benton
Body length ♂	4.09	2.47	4.90	4.26	♂ + ♀ 5.76
Body length ♀	4.50	3.59	4.75	3.99	
Tail length ♂	8.57	6.55	7.53	7.88	♂ + ♀ 6.80
Tail length ♀	6.70	6.86	6.34	7.39	
Hind foot ♂	3.95	3.35	4.16	2.38	♂ + ♀ 5.18
Hind foot ♀	1.72	3.43	3.19	2.95	
Ear ♂	4.51	2.88	4.16	2.84	♂ + ♀ 5.80
Ear ♀	2.97	4.88	4.32	3.51	

SAMPLES:

Chinook.—Twenty-five specimens from two and one-half miles southeast of Chinook, Pacific County, Washington. The area is dominated by Sitka spruce and Douglas fir. Beneath the trees are thick growths of alder, salal, and bracken ferns. The soil is moist and covered with moss (W. C. Russell, MS).

Fort Clatsop.—Fifteen specimens from Old Fort Clatsop, five miles southwest of Astoria. The forest here is composed of Sitka spruce, white fir, and hemlock. There are heavy stands of alder, ferns, and brush beneath the trees and the ground is covered with moss (W. C.Russell, MS). Four mice from Cannon Beach were included in this sample.

Astoria.—Nine specimens from Astoria, Clatsop County, Oregon. This is very close to Old Fort Clatsop and is characterized by the same type of plant associations. The specimens were collected in January, 1927. The collector took them while trapping for shrews and does not think that any of them were taken from heavy woods but rather from brushy or lightly wooded pasture land (Alex Walker, personal communication). They were not included with the Fort Clatsop sample because they were quite different in appearance.

Wahkiakum.—Nine specimens; one from Skamokawa and eight from four miles east of Skamokawa in Wahkiakum County, Washington. This area is in the Transition Zone, dominated by fir and spruce. No information concerning the exact habitat from which the mice were taken is available.

Puget Island.—Six specimens. Puget Island is in the Columbia River opposite the boundary line between Clatsop and Columbia counties, Oregon. It is closest to Oregon, but is within the political boundary of Washington. The island is about one and three-fourths miles wide and six miles long. There is not as much fir and spruce on the island as on the mainland. The vegetation is primarily a thick growth of grass, ferns, rushes, salal, huckleberry, gooseberry, snowberry, evergreen blackberry, salmonberry vine, alder, maple, and a few hemlock, poplar, and spruce (Scheffer, 1940).

Cowlitz.—Fourteen specimens from along the Kalama River in Cowlitz County, Washington. At the mouth of the river where four mice were taken are fields of heavy grass, sedge, and skunk cabbage and pasture land. This is surrounded by the dominant coniferous forest. Two to six miles east of the mouth of the Kalama River along the narrow valley of the river bed where ten specimens were taken the vegetation is quite different. At least fifty percent of the forest is of deciduous trees (willows, alders, oak, and maples). Beneath all but the most dense parts grow tall grass, bracken ferns, and clumps of hazel nut. There are also dense second growths of Douglas fir and cedar (W. C. Russell, MS).

Rainier.—Eighteen specimens from the Oregon side of the Columbia River, seven miles southeast of Rainier, Columbia County. The vegetation of the area (W. C. Russell, MS) is predominantly second-growth Douglas fir with scattered cedar, maples, and alders. The undergrowth is much less dense than in Clatsop County. It is composed of ferns, grape, hazel, and raspberry. There is more open ground with grass. The specimens were taken in the timber.

Clark.—Twenty-three specimens from Clark County, Washington; three from near Amboy and twenty from near Yacolt. The country (W. C. Russell, MS) is composed of forest and cut-over land. The forest is predominantly Douglas fir. The cut-over part is mostly brush of alder, hazel, and ferns. *Peromsycus* were caught in both habitats.

Willamette.—Twelve specimens from eighteen and one-half miles northwest of Portland in Washington County, Oregon, on the south slope of the valley of the Willamette and Columbia Rivers. The land is cut over heavily. On the damper slopes the second-growth of Douglas fir and hemlock is about

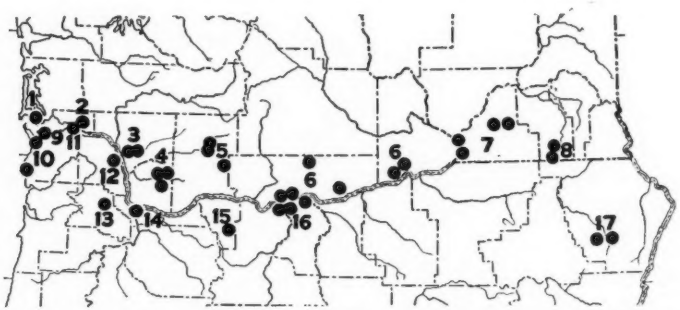
fifty to sixty feet high. The drier slopes are covered with stumps and brush. *Peromyscus* were taken along the drier slopes.

Oaks Slough Bank.—Fourteen specimens from Portland on the edge of Oaks Slough which is fed by several springs. The area is bordered by the Willamette River on the west, by the Portland metropolitan area on the north, by a crowded residential district on the east, and on the south by a mile of residential area. The bank is timbered with Douglas fir, alder, dogwood, soft maple, vine maple, hazel, and other shrubs. It has been isolated by the city of Portland since about 1880 (Stanley G. Jewett, personal communication).

Skamania.—Sixteen specimens from Skamania County, Washington. Five from Gular were taken in the forests composed of yellow pine, Douglas fir, and white fir. Eleven from near Twin Buttes, two and one-half miles southwest of Steamboat Mountain in a forest of silver pine, Sitka spruce, and western larch. (D. F. Hoffmeister, MS).

Brooks Meadow.—Fourteen specimens from Brooks Meadow, Hood River County, Oregon. It is a large meadow of several acres surrounded by forests of pine, white fir, Douglas fir, and western larch. The meadow is composed of blue grass, sedge, camas, and lupine. *Peromyscus* were not found out in the meadow, but near or in the timber. (J. E. Chattin and D. F. Hoffmeister, MS).

Klickitat-Benton.—Thirty-six specimens; nine from Satus Pass, five from Wishram, seven from Maryhill, two from near Roosevelt, ten from Glade Creek, and four from Paterson in Klickitat and Benton counties, Washington. The soil in the region is sandy. The vegetation consists of cheat grass, beard grass, thistles, sagebrush, and fields of oats and alfalfa (D. F. Hoffmeister,



A map of the Columbia River and surrounding counties in Oregon and Washington showing the localities from which the specimens were taken. The numbers represent the samples used in the text. Each number is associated with the locality or localities included in that sample. 1. Chinook, 2. Wahkiakum, 3. Cowlitz, 4. Clark, 5. Skamania, 6. Klickitat-Benton, 7. Walla Walla, 8. Stayawhile Springs, 9. Astoria, 10. Fort Clatsop, 11. Puget Island, 12. Rainier, 13. Willamette, 14. Oaks Slough, 15. Brooks Meadow, 16. Wasco-Sherman, 17. Wallowa.

MS). The uniformity of the area and the similarity of the mice in the area permitted lumping of the small samples.

Wasco-Sherman.—Twenty-four specimens; two from the mouth of the Deschutes River on the east side, twelve from the mouth of the Deschutes River on the west side, and ten from the mouth of the John Day River, in Wasco and Sherman counties, Oregon. The vegetation is predominantly sagebrush and cheat grass growing in light-colored sand. There were a few scrubby junipers about. Much of the land was under cultivation. (J. E. Chattin, MS).

Walla Walla.—Eleven specimens; two from Wallula, three from near Burbank, two from Lyons Ferry South, two from Prescott, and two from one mile west of Lamar on the Touchet River, Walla Walla County, Washington. The vegetation of the low country around the Snake River is predominantly cheat grass and sagebrush. The Touchet River area is supposedly Lower Transition, but except for the willow-poplar riparian growth, the rolling hills are covered with wheat (L. Tevis, MS).

Stayawhile Springs.—Sixteen specimens; twelve from Stayawhile Springs, and four from Godman Springs, Columbia County, Washington. The forest is composed of spruce, fir, larch, and lodgepole pine with some grass and bogs near streams (L. Tevis, MS).

Wallowa.—Fifteen specimens; eight from Wallowa Lake and seven from sixteen miles south, three miles east of Lostine, Wallowa County, Oregon. The forest here is predominantly fir with larch, lodgepole pine, spruce, and hemlock (Albert Wolfson, MS).

COMPARISON OF SAMPLES

TOTAL LENGTH AND TAIL LENGTH

(Tables IA and IB)

The variation in total length that is found in the samples is primarily due to variation in tail length. Therefore, tail length is a better measurement than total length to demonstrate the variation between the samples (Fig. 1). Mice with the greatest tail length are found in southwestern Washington. The Wahkiakum mice have the longest tails with an average of 117.2 mm. and the Chinook mice are next with an average of 109.3 mm. Farther east a very significant decrease in tail length is present. The Cowlitz sample averages 93.4 mm. The Clark sample averages longer at 100.5 mm., but this does not prove to be significant. The mean of the Skamania sample falls between the means of the last two samples. In Klickitat and Benton counties mice with much shorter tails are found. The mean for this sample is 72.4 mm. The decrease is decidedly significant. Walla Walla mice have even shorter tails than Klickitat-Benton mice, but the difference is not significant. The Stayawhile Springs sample is significantly longer in tail length than the Walla Walla sample, but not significantly longer than the Klickitat-Benton sample.

On the Oregon side of the river mice with the longest tails are again found in the extreme west. At Fort Clatsop the statistical mean is 99.9 mm. The Astoria sample, five miles northeast of Fort Clatsop is only slightly shorter in

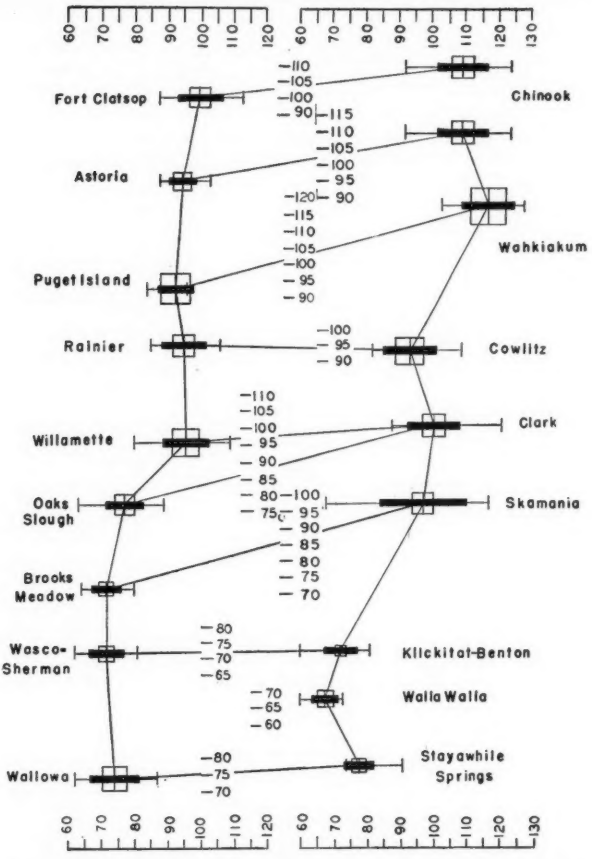


Fig. 1.—Variation in length of tail of populations of *Peromyscus maniculatus* along the Columbia River. Oregon localities are listed in the left column, Washington localities in the right. Sequence from top to bottom represents west to east. In each symbol, the observed range is indicated by the horizontal line between the short terminal vertical lines; the solid rectangle represents the standard deviation; the open square represents twice the standard error of the mean plotted both vertically and horizontally from the mean. The scales represent millimeters.

The graph is designed to allow comparison of samples from along each side of the river, and also of samples from opposite sides of the river. The samples in each vertical column are on the same scale, and differences between them are significant if the hollow squares do not overlap. Samples from opposite sides of the river which are to be compared are indicated by straight lines connecting the means. These lines intersect the common scale for these samples. For example, the Clark sample is to be compared with Willamette and Oaks Slough. It does not differ significantly from the Willamette sample because the hollow squares overlap, but does differ significantly from Oaks Slough. Chinook is represented on the graph twice.

tail length, but is significantly smaller in total length. The Puget Island mice show a closer affinity to the Oregon mice, and although they have shorter tails, they are not significantly different in tail length from any of the Oregon samples west of Portland. They are, however, significantly shorter than the Fort Clatsop mice in total length. The Rainier sample averages slightly larger than the Puget Island sample in total length and tail length. These two samples are also significantly smaller than the Fort Clatsop sample in total length. Eastward there is a highly significant decrease in the mean of the total length and tail length. There is a striking reduction present in the Oaks Slough Bank sample. These are the smallest mice in the area studied. There is a gradual increase in total length in the Brooks Meadow sample (157.8 mm.), Wasco-Sherman sample (158.8 mm.) to the Wallowa sample (165.0 mm.). The Brooks Meadow sample is slightly shorter in tail length than Oaks Slough Bank sample. The Wasco-Sherman and Wallowa samples have slightly longer tails than the Brooks Meadow sample, but are also somewhat shorter tailed than the Oaks Slough Bank sample. The Wallowa sample agrees fairly closely with the Stayawhile Springs sample.

BODY LENGTH
(Table IC)

On the Washington side of the Columbia River there is practically no significant difference in body length between any of the samples. Only the Klickitat-Benton and Walla Walla samples are significantly smaller than the sample from Chinook and the difference is slight. On the Oregon side, the Oaks Slough Bank sample is decidedly smaller than all the others. Puget Island mice are significantly smaller than Fort Clatsop mice. Brooks Meadow mice and Wasco-Sherman mice are significantly smaller than Fort Clatsop and Rainier mice. In the Wallowa sample body length has reached a size comparable to that of the Astoria and Fort Clatsop samples.

LENGTH OF FOOT
(Table ID)

Length of hind foot runs largest in the Chinook and Wahkiakum samples at about 23.5 and 23.9 mm. respectively. The Cowlitz sample drops radically to 20.9 mm. Only four specimens in this sample are as long as the minimum length found in the Chinook sample. Clark County mice are intermediate between the Cowlitz and Chinook mice. Skamania (22.78 mm.) is between Clark and Chinook and does not differ significantly from either. The three eastern samples (Klickitat-Benton, Walla Walla, and Stayawhile Springs) are almost equal in foot length with a mean of about 20.7 mm. South of the Columbia River, the samples seem to fall into two groups for this character. To the west, the means of Fort Clatsop, Astoria, and Puget Island fall between 22.0 and 22.5 mm. Eastward the means of the remaining samples fall between 20.5 and 20.9 mm.

EAR LENGTH
(Table IE)

The Chinook sample has the greatest mean for ear length, but the range is from 16 to 21 mm. Wahkiakum averages somewhat less, although the range

is only from 17 to 19 mm. in this smaller sample. Cowlitz mice again are definitely smaller. They range from 14 to 18 mm. Clark and Skamania have about the same average ear length as Wahkiakum. However, Skamania has the same range (16 to 21 mm.) as Chinook. East of this on the Washington side, the ear length is significantly smaller than all except the Cowlitz sample. On the Oregon side there is only slight variation from west to east. Rainier mice seem to have smaller ears than all the rest except those from Puget Island and Brooks Meadow.

CONDYLE-PREMAXILLAE
(Table IIB)

The Chinook sample has the greatest skull length. Wahkiakum is slightly smaller; Cowlitz is slightly smaller than Wahkiakum, and considerably smaller than Chinook. Clark and Skamania are only slightly larger, almost equal to Wahkiakum. Furthermore, Cowlitz, Clark, and Skamania, which are very heterogeneous samples for other characters, are quite homogeneous for this character. The three eastern samples, Klickitat-Benton, Walla Walla, and Stayawhile Springs, are significantly smaller than all except the Cowlitz sample. On the Oregon side the samples Fort Clatsop, Astoria, Rainier, and Willamette agree very closely. At Oaks Slough Bank the condyle-premaxillae decreases suddenly. A slightly larger average skull length is found in the Brooks Meadow sample; still larger skulls are found in Wasco-Sherman and Wallowa samples, which are not significantly smaller in skull length than the Rainier and Willamette mice.

CONDYLE-ZYGOMA
(Table IIC)

The picture presented by the condyle-zygoma is very similar to that of condyle-premaxillae. It differs primarily in that variation between the samples is less marked.

DIASTEMA
(Table IIH)

There is a sharp decrease in the mean length of the diastema from Chinook through Wahkiakum to Cowlitz samples. Wahkiakum and Cowlitz are significantly smaller than Chinook. Clark and Skamania have about the same mean as Wahkiakum, but both have extremely broad ranges. The Clark sample varies from extremely small (6.4 mm.) to as large as the largest Chinook individual (8.3 mm.). The three eastern samples are again the smallest; Stayawhile Springs is slightly larger than the other two. From Fort Clatsop to Brooks Meadow, with the exception of Oaks Slough Bank which has the smallest diastema also, there is a uniform cline from about 7.1 mm. to about 6.5 mm. Beyond this, the length of the diastema increases slightly in Wasco-Sherman mice and still further in Wallowa mice. Wallowa mice average 6.85 mm., exactly equalling the Stayawhile Springs mice.

NASAL LENGTH
(Table IIG)

Lengths of nasals show a picture very similar to that of other skull measurements. However, the character proved too variable to be of use.

ZYGOMATIC BREADTH
(Table IID)

Zygomatic breadth would seemingly be a useful measurement of the width of the skull; however, there is considerable variation within each sample. In general, the statistical means show the same pattern of variation as do the means of condyle-premaxillae. Of particular significance here is the wide range in the Cowlitz sample. The low point (12.1 mm.) is not as low as found in in the Klickitat-Benton counties sample, but is much lower than occurs in the Walla Walla sample. The high point (14.1 mm.) is almost as high as that of the largest Chinook mice. Klickitat-Benton counties sample has the greatest range of all, from about 11.4 mm. to 13.9 mm. Brooks Meadow likewise shows a high degree of variation in this character.

BULLAR WIDTH
(Table IIF)

In bullar width Chinook is largest, Wahkiakum is slightly smaller, and Cowlitz is definitely smaller than both. Clark and Skamania fall between Cowlitz and Wahkiakum. Klickitat-Benton is only slightly smaller than Skamania, whereas Walla Walla is significantly smaller than both. Stayawhile Springs mice average about the same as the Klickitat-Benton mice. From Fort Clatsop to Willamette there is a gradual, but not statistically significant, decrease in bullar width. Oaks Slough Bank sample is again quite small and out of harmony with the other samples. Brooks Meadow and Wasco-Sherman samples are only slightly smaller than the western samples and differ significantly from Fort Clatsop only. Wallowa mice are about equal in bullar width to Astoria mice and Stayawhile Springs mice.

MANDIBLE
(Table IIE)

Sharp changes are found in the mandible measurements. Wahkiakum is considerably smaller than Chinook. The difference between the means is 2.9 times the standard error of the difference. Between Wahkiakum and Cowlitz, which is still smaller, the difference between the means is 3.08 times the standard error of the difference. Clark and Skamania are again intermediate between Cowlitz and Wahkiakum. The variation from sample to sample for mandible length is consistent with variation for condyle-premaxillae. A surprisingly wide range is found in the Fort Clatsop, Brooks Meadow, and Wasco-Sherman samples, yet each has a low coefficient of variation. Cowlitz and Clark samples have equally large ranges, as might be expected, but their coefficients of variation are much higher.

PELAGE COLOR
(Table III)

Pelage color of *Peromyscus maniculatus* undergoes a definite change from west to east on both sides of the Columbia River. The darker mice are found near the coast and inland through the forest areas, whereas the lighter forms are found in the eastern sagebrush and bunch grass associations. On the Washington side of the river Wahkiakum mice are the darkest and are significantly

different from the Chinook mice. The average color tone of the Cowlitz sample is intermediate between these two for all three filters. Clark mice give readings almost identical with Cowlitz mice for the red and blue filters, and are slightly darker for the green filter. Skamania mice are lighter than Clark and Cowlitz mice. The difference is significant only for the green filter ($d/\sigma_d = 3.03$). The Klickitat-Benton and Walla Walla samples are even lighter than the Skamania sample and the differences are highly significant for the red and green filters, but not for the blue. Walla Walla mice are significantly lighter than Klickitat-Benton mice for the red filter and not significantly lighter for the other filters. The Stayawhile Springs sample is significantly darker than the Walla Walla sample for all three filters and approaches the tone of the Skamania sample in being significantly darker than the Klickitat-Benton sample for the green filter.

In Oregon there is a similar sequence of changes from dark to light. Cannon Beach mice, which were combined with Fort Clatsop mice for anatomical characters, are found to be significantly darker than Fort Clatsop mice. This difference is probably seasonal, however, as the Cannon Beach mice were collected in May and the Fort Clatsop mice were collected in August. The Astoria sample appears to be significantly darker than Fort Clatsop and Willamette samples for the green and significantly darker than Rainier and Willamette for the red and blue filters. Oaks Slough Bank mice are the darkest mice in the entire area, although not significantly darker than the Astoria mice. Continuing eastward with the Brooks Meadow sample, a very sharp change toward lighter pelage is encountered. The change is highly significant for all three filters. The Wasco-Sherman sample is even lighter than the Brooks Meadow sample, but the difference between the means is over three times the standard error of the difference for the green filter only. The Wallowa sample is significantly darker than the Wasco-Sherman sample for all three filters. It is not significantly darker than the Brooks Meadow sample and is almost identical with the Stayawhile Springs sample.

THE COLUMBIA RIVER AS A BARRIER TO DISTRIBUTION

The samples dealt with in this paper were, for the most part, chosen so that mice on opposite sides of the Columbia River could be compared. In order to discuss this problem the data already given must be considered from this viewpoint. Near the mouth of the river, the Oregon mice from Fort Clatsop, Astoria, and Puget Island are significantly smaller than the Washington mice from Chinook and Wahkiakum for total length, tail length, and hind foot. Fort Clatsop mice average considerably smaller than Chinook for skull length, condylo-zygoma, zygomatic breadth, bullar width, diastema, and mandible, but do not differ significantly from Wahkiakum in any of these measurements. Fort Clatsop mice are significantly darker than Chinook mice for the red and blue filters, but are not significantly different in tone from Wahkiakum mice. Astoria mice are decidely darker than both the Chinook and Wahkiakum mice. There is no evidence that interbreeding has taken place at the western end of the river. This broader part of the river probably acts as a definite barrier.

There is a certain similarity between the two forms of deer-mice occurring on the opposite sides of the river in that both have darker pelage, longer tails, larger ears, and longer skulls than the populations to the east of them. In spite of these similarities the two populations are morphologically distinct. It cannot be determined from my material whether these mice arose from a common stock and are diverging, or whether they arose from different stocks and are examples of convergent evolution. The important point that this situation illustrates is that even though similar environmental conditions may be responsible for selection of similar adaptations (parallel evolution), an effective isolating mechanism between two populations will maintain morphological distinctness.

There is no significant variation between the statistical means of the Rainier and Cowlitz samples for any of the characters except for a decided difference in the reflectometer readings for the green filter. The Rainier mice are slightly darker. Of particular interest here is the small ear that is present in these two samples and not in the adjacent samples. All the evidence indicates that interbreeding does take place across the river at this point, even more than at other points eastward. If this is true it may be due to the fact that these localities lie between two sharp bends in the river that might increase the possibilities of mixing of the two populations. Diffusion across the river could take place by chance occurrences such as a shifting of islands from one side to the other as the course of the river changes, by occasional freezing over of the broad slow waters as is known to happen, or by natural rafts. The only noticeable differences between the Rainier and Cowlitz samples are that the Rainier sample is darker and the Cowlitz sample shows greater variation. Selection rate for pelage color may be greater than for other characteristics. This would probably cause the mice from the partially deciduous forest river valley of Cowlitz County to take on a different shade. The greater amount of variation in the Cowlitz sample is probably due to the influence of a northern race, *austerus*, which is thought by some mammalogists to occur down to the Columbia River in this area.

Willamette and Clark samples differ significantly in total length (but not in body length or tail length alone), hind foot, condylo-basilar length of skull (but not condyle-premaxillae) diastema, and mandible. Willamette mice are smaller than Clark mice in all the above characters. There are no significant differences in color for any of the three filters. Interbreeding across the river seems less frequent between these two samples than the previous two.

The Skamania and Brooks Meadow localities are directly across the river from each other, although both are considerably further back from the river than is the case with the other samples. The Brooks Meadow sample is significantly smaller for every measurement except body length, and it is slightly paler in tone, although the reflectometer readings are not significantly different. The striking differences between these two samples could result from differences in selective factors operating on their different habitats and from a greater degree of isolation, rather than from the action of the river as a barrier.

East of the Cascades a greater similarity is found between the mice of opposite sides of the river. Wasco-Sherman mice do not differ significantly from Klickitat-Benton mice or Walla Walla mice in any of the measurements or reflectometer readings. There is a greater similarity between the Wasco-Sherman sample and the Walla Walla sample in most morphological characters and especially in pelage color. Klickitat-Benton mice are slightly larger and darker than both of these. The evidence suggests that there is intergradation across the Columbia River in this plains region, although there probably is no more crossing here than between the Willamette and Clark localities. The greater similarity in these may have resulted from a closer degree of similarity in habitat at particular localities.

From this information it seems evident that the Columbia River has been a very important barrier to the distribution of *Peromyscus maniculatus*. It has not been a complete barrier, however. Migration across the river has not been frequent enough to produce uniform populations on both sides, but it has been frequent enough to maintain general morphological similarities, except at its western end where it is a complete barrier.

CORRELATION OF MORPHOLOGICAL CHARACTERS AND HABITAT

In a general survey of the populations in this study certain broad relationships between structure and habitat appear evident. These relationships are seen when comparing the forest dwelling forms with the sagebrush and bunch grass dwelling forms. As the vegetation changes from east to west so do the characteristics of the mice. For the most part the change in structure is gradual. However, a comparison of the two extremes reveals quite a striking contrast in certain characters such as length of tail, length of ear, zygomatic breadth, bullar width, and length of diastema and mandible. These characters not only are reduced in size in the semi-arid, open country, but increase markedly in size in the heavily forested Blue Mountains. The hind foot is correspondingly reduced in the semi-arid country, but does not show the expected increase in the Blue Mountains. This character does not seem to respond to environmental changes as rapidly as some others. Grinnell (1922: 20) noted a correlation of short ears among the genera *Dipodomys, Perognathus,* and *Peromyscus* with open-type habitat and longer ears with chaparral habitat. In the present study the short ears of the mice found in the sagebrush and bunch grass habitats in Oregon and Washington support this observation. Mice from the forested regions generally have larger ears.

The length of tail seems to maintain a fairly close correlation with habitat. The longer-tailed forms are found in the west in zones of heavy forests of spruce and fir with thick undergrowth. Both the Washington mice and the Oregon mice are reputedly semi-arboreal (Dalquest, personal communication; Bailey, 1936: 183). In general the Oregon mice have shorter tails than those in Washington. On the Washington side there is a reduction in the average length of tail through Cowlitz, Clark and Skamania counties which may be partly due to the change in habitat from the spruce-fir associations to the mixed deciduous forest valley of Cowlitz County, and the white pine, silver pine,

and white fir forests of the Steamboat Mountains. The Clark County habitat from which the mice were taken is more similar to the Pacific County area and it might be noted that this sample has a slightly longer average length of tail than the other two samples. Cowlitz has the shortest average tail length of the three samples, and the habitat from which its mice came is the most diverse. In the open country of Klickitat and Benton counties, and Walla Walla County, the tail is reduced. In the Walla Walla sample the tail is most reduced in spite of the fact that some of the mice were taken from the willow-poplar associations. In the Stayawhile Springs sample the longer tail is found as would be expected if a correlation does exist with the coniferous forest. The tail is not as long as in the west, but this can be explained by the pressure of interbreeding with small-tailed populations surrounding the Blue Mountains to the south and west. A fairly uniform long tail is found on the Oregon side from Fort Clatsop through the Willamette sample. Beginning with the Oaks Slough Bank sample there is a sudden reduction of tail length which is maintained eastward through the Wallowa sample, although the latter tends to have a slightly longer tail than Brooks Meadow and Wasco-Sherman samples. The Oaks Slough Bank population is not out of the forest area and one would expect a longer tail. In fact, the marked reduction that is present in this sample in almost all cases is a bit puzzling. As far as tail length is concerned, it still substantiates the theory of long tails in forest areas because the body is also greatly reduced in size so that the tail is relatively much longer than in those samples from the open country. The Brooks Meadow sample does not conform to this picture. Mice trapped around the edge of this meadow, but still in the heavy timber, have a decidedly short tail, both absolutely and relatively. It is possible that the meadow association may have a localized effect on the length of tail but more probably this reduction is due to intergradation with the short-tailed populations of more open country to the east and south.

Bullar width and zygomatic breadth seem to be independent of the length of skull. These two characters fluctuate from sample to sample in very much the same manner as the length of tail. No obvious correlation with the habitat is present. Mandible length tends to repeat the same pattern.

In spite of the fairly constant body size in the species, there tends to be a reduction in almost all measurements of characters from west to east. This suggests that they are tied together in some manner. Sumner (1924a) has shown that various of these measurements may be genetically independent or that they may be affected generally as seems to be the case here. How much the factor of surface-volume ratio, Bergmann's Rule, (Hesse, Allee, and Schmidt, 1937: 385) reacting to differences in temperature enters into this problem is debatable. It would seem that body size itself would be more variable if this phenomenon were effective here. However, since the body measurement used is only that of length, I may have missed some actual differences in body size. Bergmann's Rule is upheld to the extent that those mice from the cooler coastal belt and the Blue Mountains are larger in almost all measurements. Allen's Rule (Hesse, Allee, and Schmidt, 1937: 389) is also upheld

in this case because the mice from the plains regions are exposed to more severe winter temperatures and possess the smaller extremities.

NATURAL SELECTION

In this discussion natural selection has been considered as the mechanism by which populations become differentiated from one another. It seems evident in studying the data in this paper that those characters which apparently are of selective value in escape from predators are more distinct from sample to sample than others. Ear length and tail length give some support to this theory. However, coat color is the best character to demonstrate the forcefulness of selection by predation. The correlation of coat color with background color has been very clearly demonstrated by Sumner (1932: 69), Benson (1932, 1933), and Dice and Blossom (1937). The importance of this is best seen in the Blue Mountain region. It has been pointed out that several mensural characters tend to increase in this region, as in the forest dwelling western mice, but that the increase usually is not very great. In coat color, however, the trend back toward the western characteristics is highly significant for all three filters. The mice here are not as dark as the Chinook or Fort Clatsop mice, but they compare more closely with the average of the Skamania mice. Coat color, therefore, would seem to be less affected by interbreeding and isolation than would length of foot, ear, or any of the other structural characters because of a greater selective pressure. An equally sound supposition is that the structural characters are affected by different selective factors in the Blue Mountains than in the coastal forests.

MICROGEOGRAPHIC VARIATION

At several points in the previous discussion rather sharp variation between two closely situated localities became apparent. It seems pertinent to examine these variations to see if special circumstances were present that could adequately explain them.

Astoria mice are consistently darker and smaller than Fort Clatsop mice. The Fort Clatsop mice were collected in August of 1940, whereas the Astoria mice were taken in January of 1927. As described earlier, the habitat around Fort Clatsop is dense forest with thick undergrowth; Astoria mice were taken from brushy or lightly wooded pasture land. This gives a basis for different selective values that might produce the small degree of variation found there. Seasonal variation in coat color could account for the difference in pelage because the mice taken in January would be in fairly fresh pelage, while those taken in August would be in worn pelage, according to the observations on *Peromyscus maniculatus gambelii* made by Collins (1923). Examination of the specimens indicates that this is the situation. Since the Astoria mice are the older specimens the differences are not likely to be due to fading in the time since they were collected.

Oaks Slough Bank mice are decidedly smaller than the Willamette mice in every measurement. It is not possible to determine the effect of isolation by

the city of Portland and the Willamette River since 1880 from the information contained in this study. A part of the acquisition of small size ("Dwarfism," Hesse, Allee, and Schmidt, 1937: 523-524) might possibly be due to isolation and the result of random fixation (Wright, 1932). It is more probable that the small size is a result of selection because in my own study and in the study of McCabe and Cowan (1945) all of the isolated populations were found to be smaller than the nearby continuous populations. Puget Island mice are smaller than those on the mainland on either side. The consistently small size of the mice in the isolated populations does not answer the theoretical qualifications of random fixation. Therefore, a selective factor common to isolated populations, possibly limited food supply, could account for the uniformly smaller size. It is apparent that the Oaks Slough Bank mice have been smaller than the Willamette mice for a long time when it is noted that all the samples from this point eastward retain similarly small proportions or only slightly larger ones. The Willamette River is certainly not as effective a barrier as the Columbia River, but that it is effective seems obvious. Quite different sets of measurements are found east and west of the river for all structural characters except foot length. The small foot of these Oregon mice begins even farther west in the Rainier sample and continues east to Wallowa. The reduction of foot length in the Rainier sample corresponds to that of Cowlitz directly across the Columbia river. Therefore, the small amount of interbreeding across either river may be enough to account for the small foot occurring so far west in Oregon. This also supports the observation earlier presented that foot length does not respond rapidly to environmental changes, e.g. in the Blue Mountains. The rates of selection for each character differ greatly. A balance between selective value and genetical flow would be established at different levels for each character and for each new locality. Foot length and ear length are more greatly altered in the Rainier sample than other characters by the influence of the Cowlitz mice. The Willamette River causes a reduction of intergradation east and west so that on the east side most mice tend toward the smaller eastern form with which they are able to interbreed most easily.

This series of samples on the Oregon side offers another illustration of the greater selecting force that determines coat color. Coat color continues dark eastward across the Willamette River through the Oaks Slough Bank sample which, according to the description of the habitat, would favor a dark pelage. Selection for dark coat color is greater than the pressure of interbreeding with lighter colored forms. The paler coat begins with the Brooks Meadow sample which is, nevertheless, darker than those from very light soil areas of sagebrush and bunch grass.

Peculiar variation is found to exist between the Chinook sample and the Wahkiakum sample. Wahkiakum mice have a slightly longer tail and hind foot; slightly smaller body, ear, condyle-premaxillae, condyle-zygoma, zygomatic breadth, bullar width, and nasal length; a significantly smaller condylobasilar length of skull, diastema, and mandible; and significantly darker pelage for all three filters. The Chinook sample was collected in August, 1940; the Wahkiakum sample was collected in June, 1920. I am not acquainted with

the exact habitat from which the Wahkiakum mice were taken and therefore do not know what environmental differences might have produced this variation.

This situation is similar to the Clatsop-Astoria condition where samples collected in 1940 from one locality differ significantly from samples of an adjacent region collected several years earlier. It hardly seems possible, however, that evolution due to a changing environment could have occurred in a period of thirteen or twenty years. It seems more likely that these are examples of microgeographic variation.

TAXONOMIC RELATIONSHIPS

On the Oregon side of the Columbia River two races of *Peromyscus maniculatus* are encountered. The darker, longer-tailed race, *rubidus*, occupies the western part of this area. The Fort Clatsop, Astoria, Puget Island, Rainier, and Willamette samples meet the description of this race in color and structural measurements (Bailey, 1936). The range of variation for these samples are: tail length, 80-113 mm.; hind foot, 19-23.5 mm.; ear, 15-19 mm; condyle-premaxillae, 23.3-25.7 mm.; zygomatic breadth, 12.2-13.8 mm.; and mandible, 15.0-17.1 mm. These figures are from samples west of the Willamette River. East of the Willamette River in the Oaks Slough Bank sample the same dark color and relatively long tail are found, but other characters are considerably modified. Ranges of variation for Oaks Slough Bank specimens are: tail length, 63-89 mm.; hind foot, 20-21 mm.; condyle-premaxillae, 22.4-23.3 mm.; zygomatic breadth, 11.8-12.6 mm.; and mandible, 14.2-15.6 mm. In spite of these smaller measurements it seems more satisfactory to include this sample with *rubidus* on basis of color and relative length of tail. Bailey (1936) indicated that *rubidus* extends slightly further east than this.

The Brooks Meadow sample is strikingly lighter colored and shorter tailed. The range of characters are: tail length, 64-80 mm.; hind foot, 19-23 mm.; ear, 15-19 mm.; condyle-premaxillae, 22.1-24.3 mm.; zygomatic breadth, 11.6-13.3 mm.; and mandible, 14.2-16.3 mm. Brooks Meadow mice are like typical *gambelii* as are the Wasco-Sherman mice. The latter are appreciably lighter in coat color. They average slightly larger than Brooks Meadow in all measurements. The ranges of structural characters for the Wasco-Sherman sample are: tail length, 62-81 mm.; hind foot, 18.5-22 mm.; ear, 15-18.5 mm.; condyle-premaxillae, 21.9-24.9 mm.; zygomatic breadth, 11.8-13.2 mm.; and mandible 14.3-16.4 mm.

Wallowa mice are considerably darker than Wasco-Sherman mice and are slightly darker than Brooks Meadow mice. They average slightly larger for most characters than both Wasco-Sherman and Brooks Meadow mice. Ranges of measurements for Wallowa mice are: tail length, 62-87 mm.; hind foot, 19-21 mm.; ear, 17-18 mm.; condyle-premaxillae, 23.0-24.8 mm.; zygomatic breadth, 12.2-13.7 mm.; mandible, 15.0-16.7 mm. Stayawhile Springs mice correspond very closely to Wallowa mice in color and structure. The ranges of measurements for Stayawhile Springs are: tail length, 74-91 mm; hind

foot, 19-21 mm.; ear, 15-19 mm.; condyle-premaxillae, 23.2-24.8 mm.; zygomatic breadth, 12.1-13.6 mm.; and mandible, 15.1-16.8 mm. Both Wallowa and Stayawhile Springs mice approach more closely the northern race *artemisiae* and should be placed in this race. Bailey (1936) and Dice (1939) agree with Osgood (1909) in calling these Blue Mountain mice *artemisiae*.

The largest mice of the Columbia Basin belong to the race *oreas*. The Chinook and Wahkiakum samples clearly fall into this race. These are dark-colored and long-tailed mice. The ranges of measurements for these two localities are: tail length, 92-128 mm.; hind foot, 22-25 mm.; ear, 16-21 mm.; condyle-premaxillae, 24.1-27.0 mm.; zygomatic breadth, 12.8-14.4 mm.; and mandible, 16.1-17.9 mm.

The status of the mice found in the area from which the Cowlitz, Clark, and Skamania samples were taken is uncertain. Further collecting and ecological study of the mice in this area is highly desirable. The problem is made complex by the relationship of *oreas* to *austerus* and *gambelii*. The northern race, *austerus*, is reported to occur in the same area with *oreas* and not to interbreed with it (Osgood, 1909: 53; McCabe and Cowan, 1945). The report by Cowan leaves little doubt that the two forms do occur together in certain areas without interbreeding. Dalquest (personal communication) thinks the range of *austerus* extends down to the Columbia River in the three counties from which the above samples were taken. The basis for such conclusions is that in his trapping he has found mice that could be placed within the range of measurements for either *oreas* or *austerus* but none that could be considered intergrades with certainty. At the outset of this study it was obvious that there was something peculiar about the mice in this region because the coefficients of variation for almost every character were very large. The Cowlitz sample has a decidedly smaller mean for almost every character than those samples immediately west or east of it. Earlier in the paper the habitat from which the specimens were taken was described as a river valley of many deciduous trees. At the mouth of the Kalama River where four specimens were taken, the forest was predominantly Sitka spruce and other conifers (W. C. Russell, MS). These four mice are darker than a few of the longer tailed forms taken further up the Kalama River. They could justifiably be called *austerus* on this basis. However, the reflectometer readings show them to be no darker than the Wahkiakum mice nor even darker than a few of the longer tailed forms taken nearby. They are very similar in appearance to a series of *austerus* from King County, Washington. All characters of these four mice run smaller than average in *oreas*: tail length 78-94 mm.; hind foot, 19-20 mm.; ear, 14-16 mm.; condyle-premaxillae, 23.1-24.9 mm.; zygomatic breadth, 12.1-12.9 mm.; and mandible, 14.6-16.0 mm. These measurements are significantly smaller than those for *oreas* as found in the Chinook and Wahkiakum samples and these specimens should certainly be called *austerus* on basis of morphological distinction. Examination of the remaining mice from this sample does not lead to such clear cut decisions. None of the remaining ones reaches the large proportions of *oreas* as found further west. They are intermediate between *oreas* and *austerus* in size, but closer to *oreas* in color. They range from 82 to

109 mm. in tail length. Osgood (1909: 63) gives the range of ten topotypes of *austerus* for tail length as 79 to 96 mm. McCabe and Cowan (1945) separate *oreas* from *austerus* at about 95 mm. Coat colors in the two races are quite distinct. Yet, the mouse with the shortest tail (82 mm.) in the Cowlitz sample is much lighter than typical *austerus* and even lighter than the average *oreas* measured here. Another specimen with a tail length of 91 mm. gives low readings for the red filter but not so low for the others. This is not as dark as the *austerus* described above, but more closely approaches them. A specimen with a tail length of 109 mm. has reflectometer readings very close to the specimen with a tail length of 82 mm. A female specimen in the series has a tail length of 99 mm.; its reflectometer readings indicate a very dark pelage, and to the eye it is only slightly lighter on the dorsal stripe than *austerus*, whereas the sides correspond almost exactly.

Foot length in this sample does not exceed 22 mm. which is considerably smaller than the foot length for the majority of *oreas*. In view of the variation of foot length in other samples studied in this paper I cannot believe that this reduction in size came about any other way than by interbreeding with a smaller footed race.

The length of the ear ranges from 16 to 18 mm.; condyle-premaxillae from 23.3 to 25.4 mm.; zygomatic breadth from 12.2 to 14.1 mm.; and mandible from 15.8 to 17.0 mm. These are mostly smaller than the average for *oreas* and strongly suggest interbreeding with a smaller race such as *austerus*. It does not appear that these reductions have been due to interbreeding with *rubidus* because this would not account for the short tail, although possibly a certain amount of interbreeding across the river takes place. Also, in *rubidus* there is a marked reddish tinge to the sides that is not present in the Cowlitz mice.

In the Clark sample range of characters is much greater (Fig. 2; Tables I-III). There are many specimens that are typical *oreas*. None of the Clark mice are as dark as *austerus*. Tail length ranges from 88 to 121 mm. The specimen with the tail length of 88 mm. is not nearly as dark as *austerus* nor does it have as much of the red to the dorsal strip and sides as do the *oreas* from the same sample. There are seven mice in this sample which more closely approach the dark color of *austerus*. Two of these are slightly lighter than the others and both have a tail length of 93 mm. Of the five darker mice, two have tail length of 101 and 102 mm., and three have tail lengths of 90 to 91 mm. Foot length of the longer tailed forms is 22 and 23 mm. Of the five shorter tailed mice it is from 20 to 22 mm. Another specimen with a tail length of 92 mm. has a considerable reddish tinge to the dorsal stripe and is generally lighter than any of the *austerus* from King County. It should be pointed out that, although the dark mice here do approach the coat color of *austerus*, they are still within the range of *oreas* from Wahkiakum and Chinook. Another possible intermediate form is a mouse with a tail length of 103 mm. and a foot length of 21 mm.

Skull characters overlap too much in *austerus* and *oreas* to separate them

satisfactorily on that basis. It would seem that the Clark sample is as much affected by interbreeding of *austerus* and *oreas* as is the Cowlitz sample. However, there are more specimens in the Clark series that would fall clearly into either *oreas* or *austerus* than at Cowlitz. It seems significant that the means for all characters are higher in the Clark series. This may be due to selection. The Cowlitz sample is from the deciduous forest river valley, whereas the Clark sample is from an area more typical of southwestern Washington. There may be habitat preferences on the part of the two races as suggested by McCabe and Cowan (1945) and others, but there is no indication of this from my information.

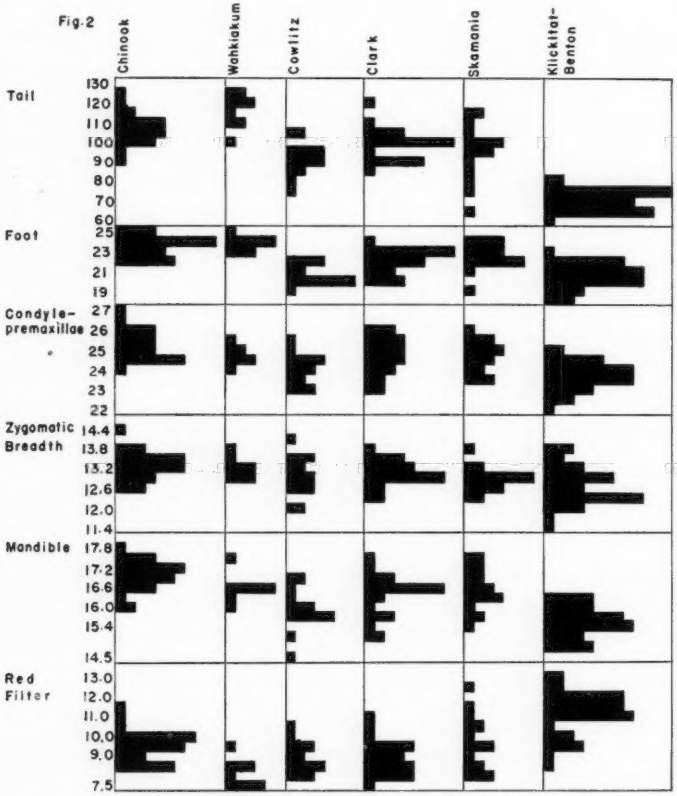


Fig. 2.—Histograms of dimensions (in millimeters) of six samples of *Peromyscus maniculatus* from the Washington side of the Columbia River arranged in geographic sequence from west to east to show trends of variation.

Peromyscus maniculatus oreas is the predominant form that occupies Cowlitz and Clark counties. At least a few *Peromyscus maniculatus austerus* are present in the same area and interbreed to a small extent with *oreas*. Closer interbreeding takes place in the Kalama River valley. The coefficients of variation in these two samples are larger for most characters than in other localities and there appears to be a greater amount of independent segregation.

In the Skamania sample there is even greater variation as the ranges extend almost from the lower limits of *gambelii* to the upper limits of *oreas* (Fig. 2; Tables I-III). Elevation for the collecting localities ranges from 2800 to 4300 feet. No mice in the series are as dark as *austerus*. Reflectometer readings for the red filter range from as dark as Cowlitz mice, but not as dark as Clark and Chinook, to exceedingly lighter than any of the western series. The lower readings for the green and blue filters are considerably above the lower readings of any of the western samples, and the upper limits are likewise higher. As can be seen from Table III the Skamania sample has the highest coefficients of variation for the reflectometer readings. In this sample it was discovered that there was a greater difference in coat color between the young adults and old adults than in other samples. The young adults do not have as much brown on the dorsal stripe as do the older mice. They are not as gray as the juvenals, especially on the sides where they are slightly brown. One specimen (M.V.Z. 87612) shows the molt between young adult and old adult in progress. The saddle across the middle of the back has completed the molt, whereas the anterior and posterior part of the back are still in the gray of the young adult. Osgood (1909) and Collins (1923) described the differences between the post-juvenal and adult pelages for *Peromyscus maniculatus*. In my material the differences are conspicuous only in the Skamania sample. Storer, Evans, and Palmer (1944) state that "in field practice we found it almost impossible to distinguish between the post-juvenal and full adult pelages."

However, the high coefficients of variation are due to the variability in the old adults and not appreciably to the young adults. Among the old adults there are a few pale, reddish individuals which register high on the reflectometer. It is these specimens which are responsible for the greater variation.

In comparing the Skamania mice with *gambelii* from Klickitat and Benton counties and with *oreas* from Chinook and Wahkiakum, considerable overlapping of the two races is found in the Skamania mice. The coat color of these mice tends to be more like that of *oreas* and *gambelii*. Because of the overlapping of color between the two races, color is not a satisfactory criterion for distinguishing them. Length of tail offers contrasting differences between the two races. The Klickitat-Benton *gambelii* range from 60 to 81 mm. in this character. The lower limit for *oreas* in the Chinook sample is 92 mm. In the Skamania series only two mice fall between these two ranges. Both of these are young adults. There is nothing distinctive in color about them that would classify them as belonging to either race. One, with a tail length of 84 mm., has a foot length of 22 mm., which is at the upper limits of the range for *gambelii*. The ear length is 19 mm. The diastema is 7.4 mm., and exceeds

all of the *gambelii* from Klickitat and Benton counties as does the mandible (16.8 mm.). The condyle-premaxillae (24.9 mm.) is also at the upper limit for *gambelii*. The other intermediate form has a tail length of 67 mm. and is far below the range for *oreas* from Chinook and Wahkiakum samples; mandible, 15.8 mm., is slightly below the lower limits for *oreas*.

There are only two mice in this sample whose tail lengths are in the range for *gambelii*. They are typical *gambelii* in all other characters. Among those mice whose tail lengths are in the range of *oreas* there are several with other characters below the range of *oreas* based on the Chinook and Wahkiakum samples. One specimen has a zygomatic breadth of 12.5 mm.; this is below the range of the Chinook and Wahkiakum samples. Two very long-tailed forms have zygomatic breadths at the lowest limits for *oreas*. This is almost average for *gambelii*. There are two specimens with condyle-premaxillae lengths of 23.7 and 23.9 mm., whereas in the Chinook and Wahkiakum samples there is only one specimen as small as 24.1 mm. for this character. The rest are above 24.4 mm.

The *oreas type* is predominant in this sample. This may be due to the forest habitat which should favor this race. The mice in this sample should probably be considered as intergrades. It is obvious, however, that intergradation is no more apparent in this heterogeneous sample than it is in the Cowlitz and Clark samples. Osgood (1909) does not indicate interbreeding of *oreas* and *gambelii* in this area but does indicate its taking place further north. Dalquest (personal communication) considers *oreas* and *gambelii* to be uniform interbreeders in this area and east of it.

The subspecies *oreas* occupies a peculiar relationship within the species *Peromyscus maniculatus*. It apparently interbreeds, but not freely, with *austerus* and *gambelii*. In the areas where interbreeding takes place, Clark, Cowlitz, and Skamania counties, individuals of the typical parent stock are more common than the intermediates. It appears as if interspecific hybridization were taking place and many of the offspring were partly or wholly sterile.

To complete the classification of the samples according to races, it needs only to be restated that the Klickitat and Benton counties mice are very good *gambelii*. Mice from the Walla Walla sample are also clearly *gambelii*.

SUMMARY

A statistical analysis of structural characters and reflectometer readings of coat color was conducted for several series of *Peromyscus maniculatus* taken along opposite sides of the Columbia River in Washington and Oregon.

On both sides of the river these mice were found to range from dark-colored, long-tailed forms in the west to light-colored, short-tailed forms in the east. On the Oregon side there was fairly uniform intergradation between the two extremes, although different characters were affected at different localities and by different factors. On the Washington side the change reached the

same end but intergradation between the two extremes was much more irregular.

The Columbia River is a complete barrier to *Peromyscus* at its western portions, but from Cowlitz County eastward it seems to permit a small amount of migration across it. This migration is enough to produce considerable similarity between the two opposite populations, especially in characters with low selective values.

A general correlation of certain characteristics with changes in habitat was found to exist. The dark-colored, long-tailed, long-eared forms were almost always found in thick forest areas. Light-colored, short-tailed, short-eared forms were found to occupy the open bunch grass or sagebrush habitat. There were exceptions to this generalization which could possibly be explained on the basis of gene flow versus selective pressure.

The study showed that each sample population differed in some degree from every other sample, and that there was a wide range of variation within each of the races as established under the present classification. Nevertheless, there seems to be no reason for breaking these races into smaller units. The samples in this study can be classified within the broad boundaries of the races: *rubidus, gambelii, oreas, austerus,* and *artemisiae. Oreas* was found to occupy the same area as *austerus* and *gambelii* in certain localities. On the basis of several specimens that did not fall clearly within any one race it was suggested that *oreas* behaved as a distinct species toward *austerus* and *gambelii* with intermittent hybridization taking place.

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TABLE I A
Total length (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	20	189-217	203.9 ± 1.6	7.13	3.50
Wahkiakum	9	198-217	205.8 ± 2.5	7.61	3.70
Cowlitz	14	156-202	184.8 ± 3.4	12.80	6.93
Clark	23	175-213	193.0 ± 1.6	7.90	4.09
Skamania	16	151-214	187.3 ± 4.1	16.52	8.82
Klickitat-Benton	36	144-179	160.2 ± 1.3	7.47	4.66
Walla Walla	10	148-165	157.3 ± 1.9	6.04	3.84
Stayawhile Springs	15	162-184	170.1 ± 1.7	6.58	3.87
Fort Clatsop	19	180-215	197.98 ± 2.1	9.32	4.71
Astoria	9	180-193	185.6 ± 1.6	4.88	2.63
Puget Island	5	173-185	179.3 ± 2.5	5.66	3.10
Rainier	17	172-201	188.3 ± 1.6	6.52	3.47
Willamette	12	175-197	184.7 ± 2.3	7.81	4.23
Oak Slough	14	142-165	154.8 ± 1.7	6.43	4.15
Brooks Meadow	14	142-172	156.8 ± 2.2	8.14	5.19
Wasco-Sherman	24	141-177	158.8 ± 1.5	7.56	4.76
Wallowa	15	141-191	165.0 ± 2.9	11.30	6.80

TABLE I B
Tail length (in millimeters)

	Number	Range	Mean	Deviation Standard	Coeff. of variation
Chinook	20	92-124	109.3 ± 1.7	7.41	6.78
Wahkiakum	9	103-128	117.2 ± 2.7	8.06	6.88
Cowlitz	14	82-109	93.4 ± 2.2	8.04	8.61
Clark	23	88-121	100.5 ± 1.7	7.94	7.90
Skamania	16	68-117	97.3 ± 3.2	12.98	13.35
Klickitat-Benton	36	60- 81	72.4 ± 0.8	4.92	6.80
Walla Walla	10	60- 73	67.7 ± 1.2	3.90	5.76
Stayawhile Springs	15	74- 91	77.8 ± 1.1	4.92	5.68
Fort Clatsop	19	89-113	99.9 ± 1.5	6.58	6.59
Astoria	9	88-103	94.6 ± 1.4	4.12	4.35
Puget Island	5	84- 96	92.5 ± 2.3	5.10	5.51
Rainier	17	85-106	95.0 ± 1.6	6.74	7.09
Willamette	12	80-109	95.7 ± 2.1	7.12	7.44
Oak Slough	14	63- 89	77.1 ± 1.6	5.82	7.55
Brooks Meadow	14	64- 80	71.5 ± 1.2	4.39	6.14
Wasco-Sherman	23	62- 81	71.7 ± 1.1	5.24	7.31
Wallowa	15	62- 87	74.1 ± 1.9	7.46	10.07

TABLE I C
Body length (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	85-107	94.5 ± 1.1	5.60	5.92
Wahkiakum	9	72-106	88.9 ± 3.4	10.08	11.33
Cowlitz	14	86-102	91.4 ± 1.6	6.04	6.61
Clark	23	84-101	92.4 ± 0.8	3.80	4.11
Skamania	16	77-100	89.9 ± 1.7	6.64	7.39
Klickitat-Benton	36	75-102	87.9 ± 0.8	5.06	5.76
Walla Walla	11	80- 95	89.4 ± 1.4	4.50	5.03
Stayawhile Springs	16	86-102	92.3 ± 0.9	3.74	4.05
Fort Clatsop	19	88-104	94.8 ± 1.0	4.28	4.51
Astoria	9	85- 98	90.5 ± 1.3	4.00	4.42
Puget Island	6	83- 91	56.2 ± 2.1	5.12	5.94
Rainier	18	83-105	93.3 ± 1.4	5.86	6.28
Willamette	12	82-100	89.3 ± 1.3	4.36	4.88
Oak Slough	14	71- 80	77.1 ± 0.9	3.50	4.54
Brooks Meadow	14	75- 89	83.6 ± 1.4	5.06	6.05
Wasco-Sherman	24	75-100	86.9 ± 1.2	6.06	6.97
Wallowa	15	79-104	90.8 ± 1.9	7.30	8.04

TABLE I D
Foot (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	22- 25	23.48 ± .21	1.03	4.36
Wahkiakum	9	23- 25	23.89 ± .22	0.66	2.78
Cowlitz	14	19- 22	20.89 ± .26	0.97	4.65
Clark	23	20- 24	22.00 ± .25	1.18	5.36
Skamania	16	19- 24	22.78 ± .32	1.27	5.57
Klickitat-Benton	36	18- 23	20.79 ± .18	1.08	5.18
Walla Walla	11	19- 21.5	20.56 ± .21	0.70	3.39
Stayawhile Springs	16	19- 21	20.69 ± .19	0.77	3.71
Fort Clatsop	19	21- 23.5	22.43 ± .16	0.69	3.09
Astoria	9	21- 23	22.25 ± .19	0.56	2.52
Puget Island	6	21.3-22.5	22.00 ± .21	0.51	2.32
Rainier	18	20- 22	20.89 ± .17	0.74	3.52
Willamette	12	19- 22	20.67 ± .25	0.85	4.11
Oak Slough	14	20- 21	20.61 ± .12	0.46	2.23
Brooks Meadow	14	19- 23	20.43 ± .26	0.96	4.68
Wasco-Sherman	24	18.5- 23	20.73 ± .20	0.97	4.68
Wallowa	15	19- 21	20.55 ± .18	0.68	3.30

TABLE I E
Ear (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	16- 21	19.28±0.24	1.23	6.37
Wahkiakum	9	17- 19	18.47±0.24	0.71	3.83
Cowlitz	14	14- 18	16.43±0.26	0.96	5.85
Clark	23	17- 20	18.52±0.19	0.88	4.74
Skamania	16	16- 21	18.44±0.31	1.22	6.63
Klickitat-Benton	36	15- 19	17.14±0.17	0.99	5.80
Walla Walla	4	16- 17	16.50±0.24	0.47	2.85
Stayawhile Springs	16	15- 19	17.00±0.22	0.87	5.09
Fort Clatsop	19	17- 19	18.00±0.17	0.72	4.03
Astoria					
Puget Island	6	16.4- 19	17.58±0.44	1.07	6.07
Rainier	18	15- 18	16.72±0.17	0.73	4.37
Willamette	11	16- 19	17.91±0.27	0.90	5.03
Oak Slough					
Brooks Meadow	14	15- 19	17.22±0.25	0.92	5.32
Wasco-Sherman	24	15-18.5	17.60±0.19	0.91	5.22
Wallowa	7	17- 18	17.98±0.18	0.48	2.66

TABLE II A
Condyle-basilar (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	21.8-24.1	22.71±0.13	0.63	2.71
Wahkiakum	7	21.7-22.6	22.25±0.15	0.38	1.72
Cowlitz	14	20.2-22.4	21.55±0.16	0.62	2.85
Clark	23	20.3-23.3	22.05±0.18	0.86	3.88
Skamania	16	20.3-23.0	21.92±0.18	0.74	3.35
Klickitat-Benton	36	19.5-22.0	20.92±0.11	0.63	3.13
Walla Walla	11	20.2-21.5	20.89±0.12	0.41	1.95
Stayawhile Springs	12	20.4-21.8	21.10±0.13	0.44	2.07
Fort Clatsop	19	20.7-23.7	21.93±0.01	0.44	2.01
Astoria	9	21.2-22.1	21.65±0.13	0.40	1.85
Puget Island					
Rainier	18	20.7-22.1	21.36±0.09	0.39	1.83
Willamette	12	20.6-22.5	21.27±0.13	0.44	2.08
Oak Slough	8	19.5-20.2	19.83±0.09	0.24	1.22
Brooks Meadow	14	19.0-21.6	20.34±0.20	0.74	3.62
Wasco-Sherman	24	19.2-21.7	20.80±0.13	0.65	3.14
Wallowa	13	20.0-22.0	21.36±0.22	0.78	3.66

TABLE II B
Condylo-premaxillae (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	24.5-27.0	22.55±0.14	0.71	2.78
Wahkiakum	7	24.1-25.7	24.94±0.21	0.55	2.21
Cowlitz	14	23.1-25.4	24.25±0.18	0.66	2.74
Clark	22	23.5-26.1	24.92±0.20	0.92	3.70
Skamania	16	23.6-26.1	24.88±0.18	0.71	2.85
Klickitat-Benton	36	22.5-25.3	23.93±0.11	0.68	2.84
Walla Walla	11	22.8-24.7	23.72±0.18	0.61	2.56
Stayawhile Springs	12	23.2-24.8	23.97±0.16	0.54	2.26
Fort Clatsop	19	23.3-25.7	24.80±0.13	0.56	2.27
Astoria	9	23.8-25.0	24.34±0.13	0.40	1.64
Puget Island					
Rainier	18	23.4-25.4	24.26±0.13	0.55	2.26
Willamette	12	23.3-25.7	24.22±0.16	0.57	2.35
Oak Slough	8	22.4-23.3	22.78±0.09	0.24	1.07
Brooks Meadow	14	22.1-24.0	23.04±0.17	0.65	2.83
Wasco-Sherman	24	21.9-24.9	23.73±0.14	0.67	2.82
Wallowa	13	23.0-24.6	24.00±0.18	0.66	2.73

TABLE II C
Condyle-zygoma (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	17.3-19.0	18.06±0.10	0.48	2.67
Wahkiakum	7	16.9-18.2	17.63±0.18	0.47	2.67
Cowlitz	14	16.3-17.9	17.32±0.13	0.48	2.79
Clark	22	16.3-18.5	17.64±0.13	0.62	3.49
Skamania	16	16.8-18.4	17.54±0.11	0.42	2.41
Klickitat-Benton	36	16.2-18.1	17.14±0.08	0.48	2.82
Walla Walla	11	16.5-17.9	17.22±0.11	0.37	2.14
Stayawhile Springs	12	16.5-17.8	17.12±0.12	0.42	2.47
Fort Clatsop	19	16.7-18.2	17.51±0.09	0.41	2.35
Astoria	9	17.0-18.0	17.33±0.10	0.31	1.80
Puget Island					
Rainier	18	16.6-18.2	17.24±0.09	0.39	2.26
Willamette	12	16.8-18.2	17.33±0.11	0.36	2.09
Oak Slough	8	16.0-16.8	16.35±0.09	0.24	1.47
Brooks Meadow	14	16.1-17.5	16.61±0.11	0.41	2.46
Wasco-Sherman	24	14.6-17.9	17.05±0.12	0.58	3.40
Wallowa	13	16.5-18.0	17.12±0.15	0.53	3.10

TABLE II D

Zygomatic Breadth (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	12.8-14.4	13.42±0.07	0.36	2.70
Wahkiakum	8	12.9-14.0	13.29±0.13	0.36	2.71
Cowlitz	14	12.1-14.1	13.06±0.14	0.53	4.09
Clark	22	12.5-13.8	13.17±0.08	0.38	2.86
Skamania	16	12.5-13.8	12.94±0.08	0.34	2.60
Klickitat-Benton	36	11.7-13.9	12.75±0.10	0.59	4.63
Walla Walla	11	12.3-13.4	12.77±0.11	0.35	2.77
Stayawhile Springs	13	12.1-13.6	12.97±0.11	0.41	3.14
Fort Clatsop	19	12.6-13.6	13.16±0.07	0.31	2.38
Astoria	9	12.7-13.5	13.11±0.10	0.29	2.17
Puget Island					
Rainier	17	12.4-13.8	12.95±0.10	0.40	3.08
Willamette	12	12.2-13.7	12.93±0.12	0.43	3.29
Oak Slough	10	11.8-12.6	12.19±0.08	0.25	2.03
Brooks Meadow	14	11.6-13.3	12.34±0.13	0.47	3.82
Wasco-Sherman	24	11.8-13.2	12.64±0.07	0.34	2.68
Wallowa	13	12.2-13.7	12.86±0.10	0.37	2.90

TABLE II E

Mandible (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	16.2-17.9	17.11±0.08	0.37	2.19
Wahkiakum	8	16.1-17.5	16.65±0.14	0.39	2.31
Cowlitz	14	14.6-17.0	15.91±0.20	0.74	4.65
Clark	22	15.3-17.6	16.44±0.13	0.61	3.71
Skamania	16	15.6-17.5	16.56±0.15	0.58	3.52
Klickitat-Benton	36	14.8-16.6	15.63±0.08	0.48	3.07
Walla Walla	11	15.0-16.1	15.49±0.10	0.33	2.14
Stayawhile Springs	13	15.1-16.8	15.94±0.15	0.54	3.38
Fort Clatsop	19	15.9-17.1	16.40±0.12	0.51	3.09
Astoria	9	15.8-16.8	16.18±0.12	0.36	2.24
Puget Island					
Rainier	18	15.0-16.7	15.78±0.09	0.37	2.31
Willamette	12	15.0-16.7	15.67±0.06	0.21	1.35
Oak Slough	10	14.2-15.6	15.03±0.13	0.39	2.62
Brooks Meadow	14	14.2-16.3	15.26±0.15	0.57	3.72
Wasco-Sherman	24	14.3-16.4	15.57±0.05	0.23	1.45
Wallowa	13	15.0-16.7	15.82±0.15	0.53	3.36

TABLE II F
Bullar width (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	10.8-11.6	11.22±0.05	0.26	2.28
Wahkiakum	7	10.9-11.3	11.07±0.05	0.15	4.16
Cowlitz	14	10.3-11.2	10.79±0.08	0.28	2.59
Clark	22	10.5-11.3	10.92±0.05	0.25	2.27
Skamania	16	10.6-11.4	10.98±0.06	0.25	2.30
Klickitat-Benton	36	10.2-11.3	10.85±0.05	0.30	2.79
Walla Walla	11	10.3-10.9	10.62±0.06	0.20	1.92
Stayawhile Springs	12	10.2-11.3	10.88±0.08	0.27	2.46
Fort Clatsop	18	10.4-11.3	10.93±0.05	0.23	2.07
Astoria	9	10.5-11.5	10.83±0.08	0.23	2.16
Puget Island					
Rainier	18	10.1-11.3	10.79±0.08	0.32	2.95
Willamette	12	10.2-11.5	10.73±0.09	0.31	2.93
Oak Slough	9	10.0-10.6	10.33±0.06	0.19	1.83
Brooks Meadow	14	10.2-10.9	10.63±0.06	0.21	1.96
Wasco-Sherman	23	10.2-11.4	10.72±0.07	0.32	3.00
Wallowa	13	10.3-11.2	10.82±0.06	0.21	1.96

TABLE II G
Nasal length (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	10.1-11.7	10.89±0.08	0.38	3.44
Wahkiakum	8	10.3-11.5	10.75±0.17	0.48	4.45
Cowlitz	14	9.3-10.9	10.26±0.12	0.43	4.18
Clark	22	9.9-11.4	10.80±0.10	0.48	4.44
Skamania	16	9.6-11.3	10.68±0.12	0.46	4.31
Klickitat-Benton	36	9.1-11.0	10.15±0.05	0.32	3.10
Walla Walla	11	9.5-10.6	9.97±0.12	0.41	4.09
Stayawhile Springs	13	9.5-11.0	10.22±0.10	0.36	3.48
Fort Clatsop	19	9.8-11.1	10.66±0.09	0.41	3.83
Astoria	9	9.6-11.1	10.33±0.18	0.53	5.08
Puget Island					
Rainier	17	9.7-10.9	10.34±0.08	0.34	3.25
Willamette	12	9.9-11.0	10.32±0.09	0.30	2.92
Oak Slough	9	9.0-10.3	9.54±0.14	0.41	4.29
Brooks Meadow	14	9.0-10.5	9.73±0.10	0.36	3.71
Wasco-Sherman	24	9.5-10.9	10.13±0.09	0.44	4.30
Wallowa	13	9.6-11.3	10.42±0.15	0.55	5.30

TABLE II H
Diastema (in millimeters)

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	7.2- 8.3	7.69±0.06	0.28	3.66
Wahkiakum	8	7.1- 7.6	7.29±0.08	0.22	2.98
Cowlitz	14	6.3- 7.6	7.09±0.10	0.35	5.00
Clark	23	6.5- 8.3	7.37±0.11	0.52	7.11
Skamania	16	6.4- 8.0	7.41±0.11	0.45	6.06
Klickitat-Benton	36	6.2- 7.1	6.71±0.04	0.26	3.91
Walla Walla	11	6.2- 7.3	6.70±0.09	0.30	4.52
Stayawhile Springs	13	6.5- 7.1	6.85±0.05	0.18	2.66
Fort Clatsop	19	6.7- 7.5	7.12±0.05	0.23	3.16
Astoria	9	6.5- 7.4	7.04±0.08	0.24	3.41
Puget Island					
Rainier	18	6.6- 7.3	6.93±0.05	0.22	3.19
Willamette	12	6.6- 7.0	6.79±0.04	0.14	2.03
Oak Slough	9	6.0- 6.5	6.31±0.05	0.15	2.30
Brooks Meadow	14	5.9- 7.0	6.46±0.08	0.31	4.80
Wasco-Sherman	24	6.1- 6.9	6.62±0.04	0.20	2.95
Wallowa	13	6.5- 7.2	6.85±0.07	0.24	3.56

TABLE III A
Red Filter

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	8.5-11.7	9.69±0.15	0.75	7.73
Wahkiakum	9	7.7-10.0	8.36±0.25	0.75	8.97
Cowlitz	14	8.0-10.8	9.07±0.22	0.83	9.09
Clark	23	7.7-11.2	9.11±0.17	0.82	9.00
Skamania	15	8.0-13.0	9.84±0.36	1.36	13.82
Klickitat-Benton	39	8.7-13.3	11.24±0.17	1.04	9.25
Walla Walla	10	11.5-13.7	12.14±0.15	0.49	4.02
Stayawhile Springs	14	8.5-11.5	10.46±0.24	0.89	8.51
Fort Clatsop	15	7.7-11.5	8.88±0.24	0.92	10.34
Astoria	9	6.7- 7.9	7.17±0.16	0.49	6.87
Puget Island					
Rainier	18	7.7-10.6	8.72±0.12	0.52	5.96
Willamette	9	7.8-10.0	8.57±0.22	0.66	7.70
Oak Slough	9	5.8- 8.3	6.80±0.27	0.81	11.91
Brooks Meadow	14	9.2-16.6	11.56±0.57	2.15	18.50
Wasco-Sherman	22	8.4-14.9	11.79±0.26	1.22	10.34
Wallowa	15	9.0-12.7	10.14±0.29	1.08	10.65

TABLE III B
Green Filter

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	6.2- 9.3	7.65±0.15	0.75	9.81
Wahkiakum	9	6.2- 7.9	7.07±0.18	0.55	7.78
Cowlitz	14	6.7- 8.9	7.69±0.17	0.64	8.38
Clark	23	6.2- 8.0	7.27±0.11	0.53	7.29
Skamania	15	6.9-10.5	8.15±0.27	1.06	13.01
Klickitat-Benton	39	8.0-11.4	9.48±0.12	0.73	7.74
Walla Walla	10	9.3-11.4	10.20±0.25	0.79	7.75
Stayawhile Springs	14	7.0- 9.6	8.41±0.21	0.77	9.16
Fort Clatsop	15	6.4- 8.2	7.24±0.15	0.59	8.15
Astoria	9	5.4- 7.1	6.20±0.17	0.52	8.39
Puget Island					
Rainier	18	5.4- 8.1	6.77±0.12	0.55	8.18
Willamette	9	6.3- 7.8	7.00±0.16	0.49	7.00
Oak Slough	9	5.2- 6.3	5.58±0.13	0.39	6.99
Brooks Meadow	14	7.2-11.2	9.04±0.26	0.98	10.84
Wasco-Sherman	22	7.0-11.9	10.18±0.21	0.98	9.63
Wallowa	15	7.0-10.0	8.31±0.23	0.99	11.91

TABLE III C
Blue Filter

	Number	Range	Mean	Standard Deviation	Coeff. of variation
Chinook	25	5.8- 7.8	6.64±0.09	0.46	6.93
Wahkiakum	9	5.3- 6.5	5.78±0.14	0.41	7.09
Cowlitz	14	5.6- 7.4	6.41±0.14	0.52	8.11
Clark	23	5.8- 7.4	6.51±0.11	0.54	8.29
Skamania	15	6.2- 9.3	7.25±0.25	0.97	13.38
Klickitat-Benton	39	6.1- 9.2	7.65±0.12	0.73	9.54
Walla Walla	10	7.4- 9.9	8.14±0.21	0.67	8.23
Stayawhile Springs	14	6.4- 8.6	7.16±0.16	0.58	8.10
Fort Clatsop	15	4.9- 6.3	5.47±0.10	0.39	7.13
Astoria	9	4.1- 6.3	4.98±0.20	0.59	11.85
Puget Island					
Rainier	18	5.3- 7.0	6.04±0.09	0.40	6.59
Willamette	9	5.7- 6.5	6.00±0.12	0.35	5.83
Oak Slough	9	4.6- 5.9	4.96±0.14	0.41	8.28
Brooks Meadow	14	6.9- 9.3	8.01±0.18	0.68	8.49
Wasco-Sherman	22	5.9- 9.9	8.36±0.16	0.75	8.97
Wallowa	15	6.1- 8.3	7.27±0.14	0.55	7.56

Notes on the Mating of Some Zonitoides (Ventridens) Species of Land Snails[1]

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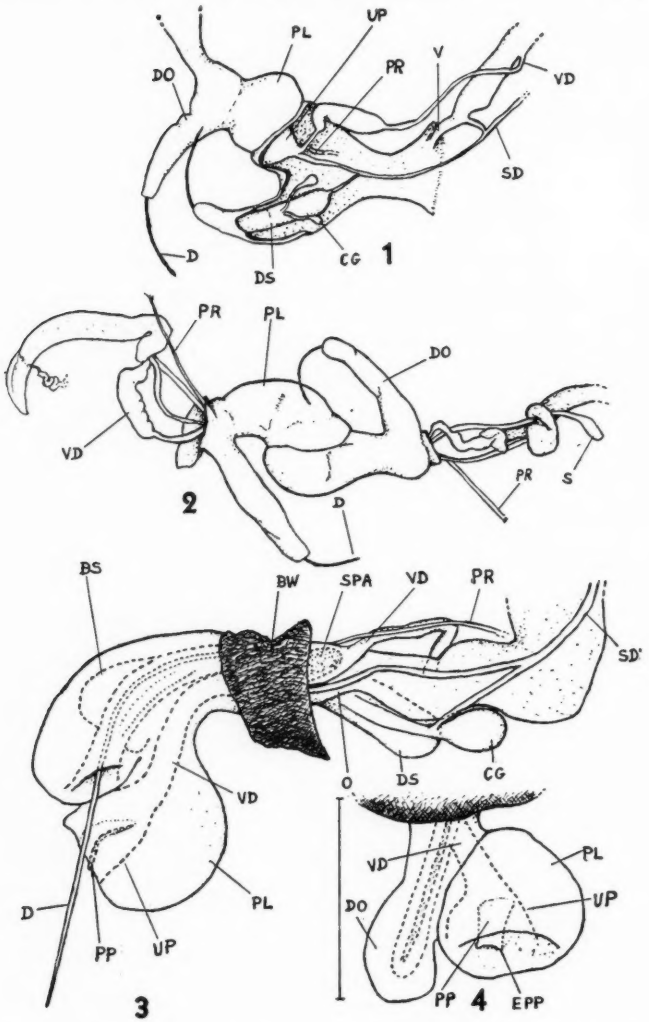
These data are presented now, despite incompleteness, because of their implications on the functional relationships of the various sex organs exhibited by gastrodontin landsnails, and because of their bearing on the problem of hybridism in these snails. Similar methods were used in obtaining these data as have been used in studies of polygrid snails (Webb, 1947). Caged specimens only were studied, and the matings observed occurred under artificial conditions. In nomenclature, I have followed Pilsbry's *Land Mollusca of North America,* vol. 2, part 1. Without the accessibility to most of the literature on these zonitid snails supplied by this work, the present paper would not have been attempted in the spare-time available. Figures 1, 2, 6, and 7 are somewhat diagrammatic, and have been drawn (greatly enlarged) from the freshly dissected material. Figures 3, 4, and 5 are from projection-tracings (inversion uncorrected) of material mounted in Canada balsam, and the scale-lines represent 2 mm. The anatomy illustrated by fig. 5 is somewhat distorted from the effects of its former preservation in sodium silicate.

Three species of *Ventridens* are discussed in this paper; while an unidentified species *(V. dimissus brittsi?* (Pilsbry), from Mt. Magazine, Ark.?) is also briefly mentioned. The basis of the presented data is: 5 specimens and 3 matings of *Ventridens ligera* (Say), 2 specimens and 1 mating of *V. suppressus* (Say), 2 specimens and 1 mating of *V. intertextus* (Binney), and 1 mating of the unidentified *Ventridens* with a specimen of *V. ligera.*

Except for several instances of voluntary matings among group-cultured specimens, all of the matings observed have been planned matings. For this reason mating periodisms would have been unlikely of observation; and, indeed, no evident periodicity of mating was noted. However, one volutary mating occurred in the daytime shortly after the dry cage had been remoistened, and this might be interpreted as indicating a possible correlation of matings to periods of rainfall or high humidity. In this regard concerning *Zonitoides arboreus* (Say), Bartsch & Quick (1926) have written: "Most of the specimens found conjugating were discovered in the early morning on moist days." Unfortunately I have not observed any matings of *Ventridens* species in the field, and any who note such should add to our knowledge by publishing detailed descriptions, by obtaining photographs, or by securing mating-anatomies.[2]

1 Read at the June 1947 meeting of the American Malacological Union.

2 In the field mating-anatomies may be secured by crushing the shells but not the extruded organs, and plunging the disabled snails into some preservative as 75% alcohol.



Figs. 1-4. *Ventridens* spp.—1. *V. ligera* (Say), W. Fk. of White Riv., just S. of Marion-Johnson county lines, Ind. One of the mating-anatomies is shown dissected; 2. *V. intertextus* (Binney), Hancock Co., Ind., about 3 mi. E. of Cumberland and ½ mi. S. A pair of mating-anatomies; 3. *V. suppressus* (Say), Dauphin Co., hills above Rockville, Pa. A mating-anatomy; 4. *V. suppressus*. The other mating-anatomy. Note the projecting edge of the penis-plate. See fig. 5 for abbreviations.

In securing the planned matings, a pair or group of species-isolation-cultured specimens were placed in a dampened container; and, if two specimens seemed to be courting, any extra snails were removed. When it was found that a swollen or dilated genital-pore was a reliable index of mateability, it was possible to select mateable couples sometimes at will. The most extreme sign of mateability is that of the sex-organs extruded as in courtship. Soaking the specimens in water seems also of use in helping to induce matings, and this procedure frequently was followed.

COURTSHIP

To obtain courtship data, considerable vigilance is sometimes needed; because often mateable specimens will rest for hours and then, when the observer relaxes, will be found involved in the later stages of mating upon his return perhaps only a short time later. Possibly for this reason adequate courtship data are available only for *ligera;* although the one mating observed of *V. suppressus* supplies some additional information.

In *ligera* one snail has been noted to instigate courtship by crawling onto the spire of the other snail, but such behavior may not be essential. The rest of the courtship and mating involved a more-or-less head-on position; I have considered the contiguity of the penis-lobes as indicating mating. The dart-organ[3] performs the major function of sexual excitation, and is exserted before or shortly after the onset of courtship. The rest of the penis may, or may not, be exserted at this time. When the entire organ is exserted, it appears as a stemmed, medially deeply-bifurcated body—the upper (outermost) finger-like lobe being the exserted dart-organ containing the protrusile dart, and the slightly-smaller lower lobe being the incompletely turgescent remainder of the penis. Either lobe can be retracted separately of the other. When functioning, the dart-organ is usually brought into contact with the other snail, and the bare dart is then protuded beyond the sheath-like part either into the tissues of the mate or otherwise. The protuded part of the dart commonly extends more than 6 mm. beyond the orifice of the dart-organ. While functioning the dart is rapidly jabbed inwards and outwards, and generally each successive jab is not preceded by the complete re-sheathment of the dart. The intra-dart-organ tubules exhibit convulsive movements while the dart is jabbing. At times a slow steady beating within the exserted penis adjacent to the genital pore has been observed; perhaps the coronal glands are causative of this action. The areas most commonly probed and pierced by the dart are the sides of the foot, the region adjacent to the genital pore, and the extruded organs.

In addition to the dart, biting has also been observed in the courtship of *V. ligera* and of *V. intertextus*. My impression is that biting is employed more in reaction to the dart than as an additional mode of sexual stimulation. Occasionally a dart-wounded animal has been observed to retaliate by biting the body or extruded organs of the offending animal, or by temporarily moving away.

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3 Definition: The unit composed of dart-sac, dart, and associated structures which functionally is distinct from the penis.

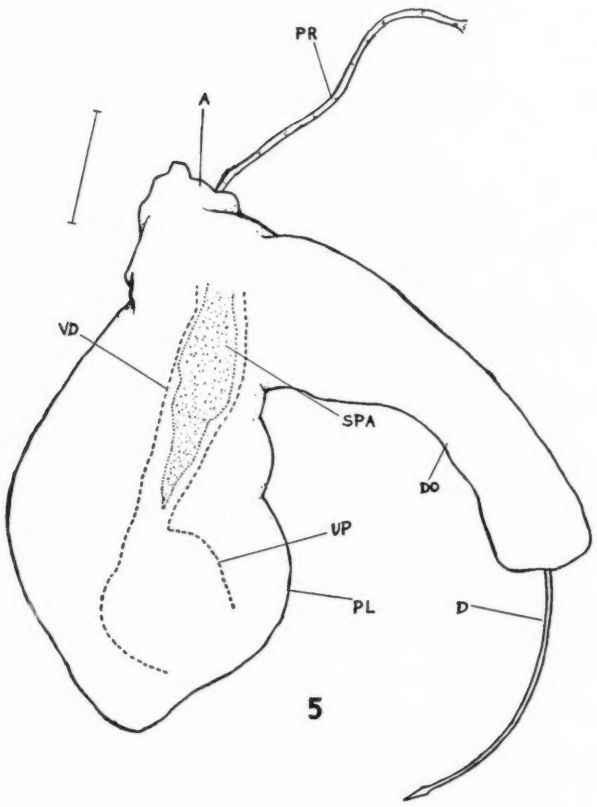


Fig. 5.—*V. intertextus*, Hancock Co., Ind., one mating-anatomy showing vas deferens and contained spermaotphore.

Explanation of symbols: A—Atrium, AG—Albuminiferous gland, BS—Bladder-like structure, BW—Body wall, CG—Coronal gland, D—Dart, DO—Dart-organ, DP—Dissecting pin, DS—Dart sac, EP—Ejaculatory pore, EPP—External penis-plate, O—Oviduct, PIL—Pilaster, PL—Penis-lobe, PP—Penis-plate, PR—Penis retractor, S—Spermatheca, SD—Spermathecal duct, SPA—Spermatophore, U—Uterus, UP—Upper penis, V—Vagina, VD—Vas deferens.

The seeming objective of the courtship maneuvers is to induce the extrusion of the sex-organs, and to bring the extruded penis-lobes into contact. All locomotive movements of the animals usually cease after the penis-lobe of one snail is brought into contact with that of the other. The penis-lobe attains maximum turgidity at this time. While still capable of being fully turgesced, the dart-organ functions less and less frequently unless the mate-animal attempts disunion too soon.

The courtship behavior of *suppressus*, so far as revealed by observations of a single mating, differs apparently in two regards from that of *ligera*. Instead of a preliminary phase of one snail mounting the other's shell, one snail pursued the other. In this the leading snail moved in a circle while the other followed, keeping its mouthparts pressed against the tail-tip of the snail in front. Biting or gnawing by the pursuing snail was not seen but may have been occurring. The second difference is mainly anatomical. Whereas the extruded organs in *ligera* are deeply forked with finger-like branches, in *suppressus* the terminal lobes of the feebly bifurcated organ are shorter than the basal stem. The dart-organ consequently is much less motile than in *ligera*. In the extruded organs of courting animals the bifurcation is even less evident, and the penis-lobe is almost similar to the dart-organ lobe.

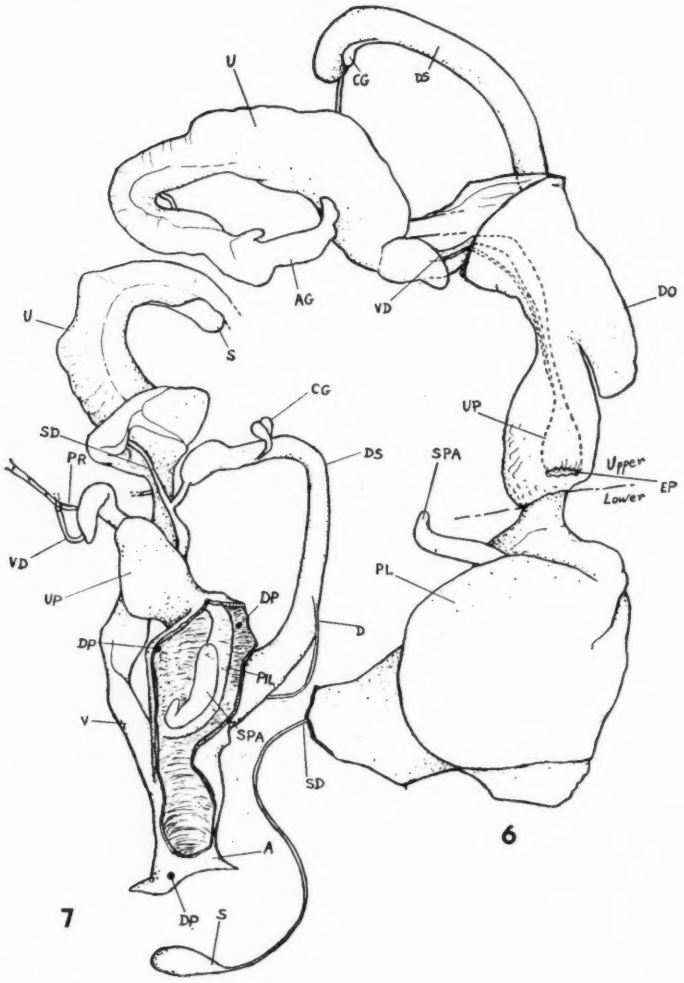
As in *ligera*, contiguosity of the penis-lobe indicates mating, and the end of courtship; and the snails were head-on (somewhat obliquely so) after the early part of courtship. One pivoting act was observed.

Courtship actions have not been observed to last more than about half-an-hour in any of the species observed, and it seems likely that the entire mating process normally is completed in somewhat less than two hours.

SPERMATOPHORE-TRANSFER (the completion of mating)

The conclusion of the mating process has been observed only in a mating between a Ft. Smith, Ark. specimen of *V. ligera*, and the unidentified *Ventridens*. However, an almost completed mating has been observed in which the spermatophore-ejection stage had been reached, and one of the snails had seemingly started to disengage when I disrupted the process to verify the observations by getting mating-anatomy material. The entire mating-process of *ligera* is illustrated, however, by material. The available mating-anatomies of the other two species studied exhibit the first stage condition, and the relationships of the organs imply that they have an almost identical mating-process.

In what is here termed the first stage of the mating process, the penises are merely contiguous by their ejaculatory-pore-bearing surface, and the spermatophore or spermatophores yet have not descended to the exterior. In most mating-anatomies the organs are so firmly adherent that it is impossible to separate them and so to examine the ejaculatory-pore-bearing surface. Fortunately (fig. 4) the mating-anatomies of the *V. suppressus* were separable and confirm the suspected absence of any orifice on the penis-lobe except the ejaculatory pore. Note particularly (fig. 4) how one edge of the penis-plate is exposed as a knife-like tooth. Although probable, it will yet have to be determined if the penis-plate of *ligera* is situated similarly.



Figs. 6, 7. *Ventridens ligeria* (Say).—6. Upper mating-anatomy from Harrisburg, Pa., specimen; lower anatomy from a Ft. Smith, specimen. The Ft. Smith specimen bears a spermatophore from the other snail; 7. *V. ligera*, Post-mating anatomy showing secured spermatophore. This Ft. Smith specimen had been mating with another species of *Ventridens*. See fig. 5 for abbreviations.

The next stage (fig. 6) in the process is illustrated by a Ft. Smith-Harrisburg mating wherein the discharged spermatophore was noted lodged in the penis-tissues of one animal, which itself had not ejected its own spermatophore. During this mating the one snail appeared as if it were retracting its organs preparatory to moving away. The pair of mating-anatomies obtained confirms this, and shows that the ejected spermatophore came from this specimen. In the other specimen, the penis was found to have a small hole in which the spermatophore was partially imbeded. This hole is thought to represent a rupture and wounding of the penis-tissues—a viewpoint subsequently substantiated.

The final stage is illustrated by the inter-species mating between a Ft. Smith specimen and a small, depressed species, the unidentified *Ventridens*. This pair were noted with the organs in contact with a spindle-shaped body "adhering" seemingly to the penis of the larger snail. This animal soon completely retracted the sex-organs and withdrew. The unidentified *Ventridens* continued to crawl about with exserted, deeply-forked sex-organs. The Ft. Smith specimen was then killed in boiling water and dissected. Its organs were found to be almost as in the non-mating condition except that a spindle-shaped body, similar to that formerly thought to be "adhering" to the everted penis-lobe, was found slightly imbeded at one end in a papillose, ridge-like pilaster contained in the lower penis-cavity. The removal of this body, which was obviously a spermatophore, revealed a shallow hole where the pointed end previously had been. This confirmed the earlier find—the spermatophore truely was apically embeded in the penis-tissues and its removal left a hole.

The mode of spermatophore-transfer is thus evident. The sharply-pointed tip of the spermatophore is ejected or implanted into the turgid, thin tissue of the penis of the mate-animal while the penis-lobes are contiguous, and later is conveyed into the body with the retraction of the organs. The relationship of the implanted spermatophore to the spermathecal ducts and spermatheca remain yet to be clearly determined. Presumably the spermatophore is ultimately conveyed into the spermatheca. The variation in the placement of spermatophores, as recorded by Baker (1929) for *Ventridens elliotti* (Redfield), may bear on this problem.

Another unsolved problem is the means by which the spermatophore is implanted into the penis-tissue of the mate-animal. Seemingly only the following means of penetration by the spermatophore exist: (1) by means of sharp spermatophore-tip and the force of ejection, or (2) by the incision of the mate-animal's penis by the projecting edge of the penis-plate with a similar forceful implantation of the spermatophore into the wound, or (3) by the wounding of the penis by the other animal's dart followed by the forceful implantation of the spermatophore into the wound. Possibly the process utilizes a combination of these factors. The most probable explanation seems to be that of the use of the penis-plate in eroding or piercing the penis-surface, followed by the forcible implantation of the spermatophore into the weakened or wounded tissues by means of the non-extruded, funnel-shaped, upper penis in which the penis-plate is situated. See figs. 3, and 4. No functional vagina or female-organ was found in any of the mating-anatomies

studied, the nominal vagina merely terminating duct-wise at the base of the atrium and not being involved with the extruded sex-organs.

The basic similarity to *Ventridens ligera* in sex-organ structure of most of the Gastrodontinae[4] makes it seem probable that basicly the same physiology of mating (with a like mode of spermatophore-transfer) pervails widely throughout the subfamily. Mechanically the mating process of these snails seems readily to permit of inter-species matings, and it would seem that extensive hybridism can occur unless genetic or behavioral incompatibility prevents.

MATING-ANATOMY COMPARISONS

Too few mating-anatomies were obtained to afford a clear understanding of the variation likely to be found. The following comparisons, therefore, should not be understood as indicating more than the probable forms of the organs.

Ventridens ligera and *V. intertextus*.—The mating-anatomy characteristics of these two species are much alike. The only differences noted were the greater departure from spheroidicity of the penis-lobe, and a slightly greater length of the dart in *intertextus*. In fig. 3 all structures were omitted that could not be clearly discerned in form or relationship. Note the spermatophore which was probably moving toward the ejaculatory pore when the mating animal was killed. The elongate dart-organ is about 9 times longer than wide, and is similar in these two species. The dart-sac (fig. 1) can descend completely into the cavity of the extruded parts of the penis.

Ventridens suppressus.—The entire extruded-portion of the sex-organs appears as a Y-shaped body in which one branch (penis lobe) is much more swollen than the other (dart-organ). In freshly obtained mating-anatomies, the distance from the body-wall along the axis of the dart-organ (exclusive of the protuded dart) was found to measure approximately 3¼ mm. In the two anatomies the penis-lobe varied from 1¾ to about 2 mm. in diameter. The penis-lobe appears almost perfectly spherical and bears the excentrically placed ejaculatory pore on the truncated tip. The weakly crenulated edge of the penis-plate is exposed and reaches the exterior near the ejaculatory pore. The dart-organ is about as broad as long. About half of the dart-sac is yet retained undescended into the penis in both anatomies. A strange bladder-like membranous structure (BS), of unkown significance, is present about the dart-sac in one of the specimens.

As would be expected from the smaller size of the snail, the extruded organs are much smaller in *suppressus* than in the two preceding species, and the lobes of the penis are also much less elongate.

SUMMARY

1. Data are presented on the sex-biology and anatomy of *Ventridens intertextus* (Binney), *V. ligera* (Say), *V. suppressus* (Say), and one other of uncertain identity which may have been *V. dimissus brittsi* (Pilsbry).

4 As indicated by the findings of Baker, Pilsbry, and others.

2. It was found possible to select mateable couples at will by pairing snails noted with swollen or dilated genital pores, or even with already everted sex-organs.

3. In *ligera* in the matings observed, courtship commenced with one animal mounting the spire of the other; later a more or less head-on position was attained by the snails. A variable period of reciprocal dart-probing ensued until mating followed the attainment of penis-lobe contiguosity. The dart-organ tends to become less functional as mating continues.

4. Biting during mating seemed a defense reaction against the dart. Pivoting acts were noted in several instances.

5. In the pair of *suppressus* observed, courtship differed from that of *ligera* in that one snail pursued the other, culminating in a non-reciprocal head-to-tail circling; but mating began similarly when the penis lobes became contiguous. The short dart-organ functions as in *ligera* except for its lesser motility.

6. Courtship has not been observed to last more than half-an-hour, and it seems probable that the entire mating process is completed in less than two hours.

7. Insemination is effected (in *ligera)* by the insertion of a sharply pointed spermatophore partially into the penis-tissues of the mate so that it occupies a small, shallow, hole-like wound. Three possibilities were conjectured as to the mechanics of the process.

8. The limited data available indicate that the spermatophore-transfer process is non-reciprocal.

9. If similar to that observed, the mechanics of mating may permit widespread interspecies-matings among gastrodontin landsnails. Successful spermatophore-transfer was noted to occur in a mating between dissimilar forms of *ligera* from Arkansas and Pennsylvania, and between *ligera* and a different species.

10. The only differences noted in mating-anatomy characteristics between *ligera* and *intertextus* in the limited material studied were the less rounded penis-lobe, and the longer dart in *intertextus*. The dart-sac descends completely into the everted penis in both.

11. The *suppressus* studied differed from *ligera* and *intertextus* in the greater proportinate length of the basal penis, and in the shortness of the penis-lobe, and dart-organ lobe.

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Observations on Cestodes in North American Owls with the Description of Choanotaenia speotytonis n. sp.* (Cestoda: Dipylidiinae)

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Little is known concerning the helminths parasitic in several major groups of the North American avifauna. This appears to be particularly true of the parasites of owls. Over the past few years the writer has had the opportunity of examining a fairly large series of these birds, and they have been found to be infected with several helminths of unusual interest (Chandler and Rausch, 1947).

It should be pointed out that most of the owls examined were not killed for this purpose alone, but were obtained from game farms and refuges, under conditions where their destruction was considered necessary. Strict protection of raptors, under ordinary conditions, is most desirable at all times.

This paper is concerned with two species of cestodes encountered in 85 owls, representing 10 species and sub-species, examined over the past 5 years. The birds examined were: great horned owl, *Bubo v. virginianus* (Gmelin); arctic horned owl, *Bubo virginianus subarcticus* Hoy; barn owl, *Tyto alba pratincola* (Bonaparte); screech owl, *Otus asio naevius* (Gmelin); barred owl, *Strix v. varia* Barton; short-eared owl, *Asio f. flammeus* (Pontoppidan); long-eared owl, *Asio wilsonianus* (Lesson); snowy owl, *Nyctea nyctea* (Linnaeus); saw-whet owl, *Cryptoglaux a. acadicae* (Gmelin); and burrowing owl, *Speotyto cunicularia hypugaea* (Bonaparte).

Except for the arctic horned owl, collected in Manitoba, and the burrowing owls, collected in Colorado, all the birds examined were from the North Central States area. Through the cooperation of the staff of the Wisconsin State Game Farm, at Poynette, Wisconsin, a large number of owls was secured from that area. Most of these were immature great horned owls, collected during the winter months.

Cestodes are generally uncommon in owls, as pointed out by Wolffhügel (1900), who reviewed host records previous to that time. The most common cestode occurring in owls is *Paruterina candelabraria* (Goeze, 1782). Ransom (1909) reported this species from the short eared owl; he also listed *Taenia strigis-acadicae*, superficially described by Leidy (1855), which may or may not be identical with *P. candelabraria*. According to the files of the Zoological Division of the Bureau of Animal Industry, no cestode has been recorded

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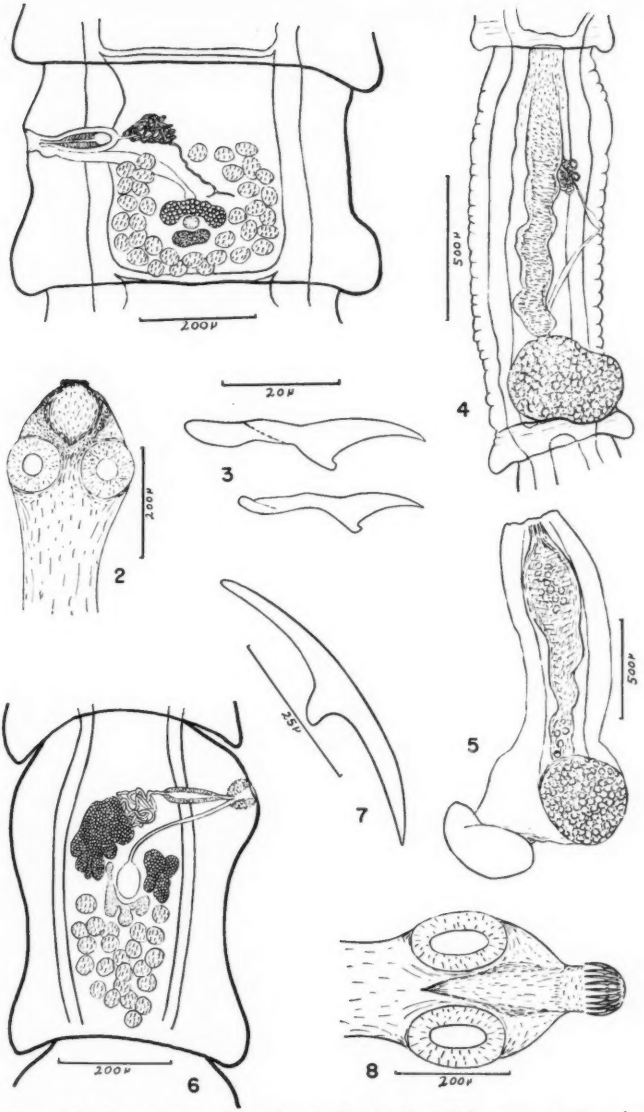

Figs. 1-5. *Paruterina candelbraria.*—1. Proglottid slightly past maturity—showing early development of uterus; 2. Scolex without rostellar hooks; 3. Rostellar hooks; 4. Gravid proglottid; 5. Shed proglottid as found in feces of the host. Figs. 6-8. *Choanotaenia speotytonis.*—6. Mature proglottid; 7. Rostellar hook; 8. Scolex.

from either the great horned owl or the burrowing owl. This information was not obtained for the other North American owl species.

I. PARUTERINA CANDELABRARIA (Goeze, 1782)

Figs. 1-5

The writer found *P. candelabraria* on 9 different occasions; 8 times in the great horned owl, and once in the barred owl. Cestodes from two screech owls, collected by Dr. F. N. Hamerstrom, in Iowa, probably belong to this species also, although poor preservation made definite specific determination impossible. In addition, the writer secured 2 immature cestodes from a snowy owl, but identifiaction could not be made.

Paruterina candelabraria was completely described by Wolffhügel (1900), but since his description is not readily accessible, it might be of some value to include here a description of this species, based on North American material.

Diagnosis.—Maximum length of strobila 300 mm; greatest width, attained in post-mature proglottids, about 1 mm in well-relaxed specimens. Mature proglottids wider than long, gradually increasing in length after maturity. Gravid proglottids decrease in width; terminal proglottids measure about 600 μ wide by 2 mm long. Longitudinal muscle fibers strongly developed and numerous. Scolex, from 213 to 284 μ in diameter, not sharply set off from neck; suckers average 102 μ in diameter. Rostellum armed with about 50 hooks, arranged in a double row. Large hooks measure from 39 to 46 μ long (average 42 μ); small hooks measure from 29 to 34 μ long (average 33 μ).

Ventral excretory canals variable in diameter, from 28 to 130 μ, with greatest diameter occurring in mid-section of segment. Transverse canals similar in diameter to narrow part of ventral canals. Dorsal canals are lateral to ventral canals; about 16 μ in dimeter.

Irregularly alternating genital pores are situated near the middle of mature proglottids. Genital ducts pass between longitudinal excretory canals. Cirrus sac, averaging 132 by 46 μ in mature proglottids, may or may not overlap ventral excretory canal. Cirrus unarmed; about 60 μ long by 10 μ in diameter. Ductus ejaculatorius somewhat coiled within cirrus sac. Vas deferens strongly coiled medial to ventral excretory canal; coils persist in gravid proglottids, where they are situated between the margin of the parauterine organ and the ventral excretory canal.

Testes ovoid; from 28 to 35 (average 31) in number, and measuring from 33 to 46 μ in diameter in mature proglottids; not all in same plane. Testes lateral and posterior to female genital organs, extending forward to level of genital pore.

Vagina opens posterior to and in same plane as cirrus sac; distal end provided with well-developed sphincter. Vagina usually dilated to about 30 μ in diameter from genital pore to level of proximal end of coils of vas deferens (about 350 μ). Function of enlargement not clear; it appears to be filled with spermatozoa in some segments. After narrowing in diameter, the vagina runs

downward to join the elongate seminal receptacle; latter is about 30 μ in diameter, and situated between ovarian lobes.

Ovary, in mature proglottids, consists of two spherical lobes up to 100 μ in diameter; situated near mid-line in posterior half of proglottid. Vitelline gland hemispherical, situated immediately behind and parallel to ovary. Uterus first seen as two lateral lobes immediately dorsal to ovary. It is often impossible to separate the two, giving the impression that the ovarian lobes become confluent and enlarged. Ovary disappears soon after appearance of uterus; vitelline gland and seminal receptacle persist longer, and gradually disappear as uterus increases in size. As proglottids become older, uterus migrates toward posterior margin; the parauterine organ, at first tortuous in outline, gradually appears from the anterior margin of the uterus and extends to the anterior margin of the proglottid. Uterus gradually assumes an ovoid shape at posterior margin of proglottid, occupying the entire space between excretory canals. Parauterine organ gradually becomes straighter toward posterior end of strobila, remaining somewhat sinuous to time proglottid is shed. In last few proglottids the gravid uterus is seen at the immediate margin, giving rise to a large, lateral projection; this enlargement is usually aporal, but may occur on either side.

Gravid proglottids break near the level of the genital pore, although they appear very weak at the level of the uterus. Very few eggs, if any, can be seen inside the parauterine organs, either in gravid or shed proglottids. Eggs very numerous, from 30 μ to 33 μ long by 20 μ to 23 μ wide (average 31 by 21 μ).

DISCUSSION

Through the kindness of Dr. Jean G. Baer, Director of the Institut de Zoologie, Université de Neuchâtel, the writer had the opportunity to examine several slides of *P. candelabraria* from European owls. These slides, from *Strix* and *Asio*, were from the collection of the late Professor Fuhrmann, from material from the Museums of Berlin and Vienna.

Since the slides were quite old, it was not possible to make out all the morphological details; however, the specimens agreed very closely with those from North American owls. As far as could be determined, the gravid uterus was relatively larger in the European specimens, and the parauterine organ was straighter. The average egg measurements were identical. On the single scolex examined, the hooks were appreciably larger than those of the American specimens. The large hooks averaged 56 μ, while the small hooks averaged 43 μ. While these are larger than usual, there is considerable variation in hook size, according to Wolffhügel (1900). The hooks were identical in shape.

Since Wolffhügel did not have available terminal or shed proglottids, he was uncertain as to the complete development of the uterus and parauterine organ. Shed segments, as removed from the feces of the host, had a very typical appearance (Fig. 5). Eggs are not ordinarily found in large numbers in the parauterine organ, and in most cases none at all could be seen. The writer had available for comparison only one other species of the genus *Par-*

uterina; this was *P. similis* (Ransom, 1909), from the yellow-billed cuckoo. In *P. similis* likewise, the eggs could not be seen in the parauterine organ.

Two other species of *Paruterina* have been recorded from owls: *P. otidis* Baczynska, 1914, and *P. angustata* Fuhrmann, 1906. Both species are readily differentiated from *P. candelabraria. Paruterina angustata* has unilateral genital pores, fewer testes (20), and is much smaller in size (50 mm); *P. otidis* has fewer testes (15), alternating genital pores, many more hooks (92), and is also of small size (20 mm).

Slides containing whole-mounts of *P. candelabraria* have been deposited in the collections of the U. S. National Museum and the Institut de Zoologie, Université de Neuchâtel.

II. Choanotaenia speotytonis n. sp.

Figs. 6-8

One of two burrowing owls collected for the writer by Mr. N. C. Negus, near Denver, Colorado, contained a large number of cestodes apparently belonging to the genus *Choanotaenia* Railliet, 1896. These represent an undescribed species. Since the cestodes were taken from viscera preserved in formalin, certain details unfortunately could not be obtained.

Diagnosis.—Strobila about 30 mm long, containing from 70 to 100 proglottids. Greatest width about 1100 μ, attained in late mature proglottids. Proglottids of relaxed strobilae longer than wide; contracted proglottids bell-shaped and strongly annulated, about 820 μ wide by 355 μ long (mature). Scolex from 270 to 290 μ in length; suckers average 156 μ in diameter. Rostellum strongly developed and armed with a single row of 18 hooks. Hooks measure from 46 to 56 μ in length (average 52 μ).

Ventral excretory canal about 23 μ in diameter; dorsal canal about 10 μ. Genital pores, situated near anterior end of proglottid, irregularly alternate. Genital ducts pass between longitudinal excretory canals. Cirrus sac usually somewhat inclined toward anterior end of proglottid; about 100 by 33 μ. Vas deferens strongly coiled, with coils extending from near end of cirrus sac to medial margin of aporal lobe of ovary. Vasa efferentia run from anterior margin of testes. Testes from 19 to 22 in number (average 20), and measure from 29 to 33 μ (average 31 μ). Testes extend on both sides forward from posterior margin of proglottid to level of posterior margin of respective ovarian lobe.

Vagina opens posterior to and in same plane as cirrus sac, and runs medially and posterior to enter large seminal receptacle (about 55 μ in diameter in mature proglottids). Ovary arranged in two separate, lobed masses; aporal mass considerably larger, and more anterior. Vitelline gland semilunar in shape; situated just posteior to seminal receptacle, and extending around it on either side forward to the posterior margin of the respective ovarian mass. Uterus sac-like; exact course of development not clear. Entire gravid proglottid filled with eggs, which appear, on frontal section to be enclosed within cells in the parenchyma. Eggs ovoid; from 46 to 53 μ long by 39 to 43 μ wide. Hooks of embryo about 16 μ long.

Host.—*Speotyto cunicularia hypugaea* (Bonaparte) (Western burrowing owl).

Locality.—Near Denver, Colorado.

Habitat.—Small intestine.

Type.—Slides containing cotype specimens have been deposited in the helminthological collection of the U. S. National Museum. Additional specimens have been deposited in the collection of the Institut de Zoologie, Université de Neuchâtel.

DISCUSSION

This cestode has been assigned to the genus *Choanotaenia* by virtue of the arrangement of the eggs in the gravid uterus, as determined by means of frontal serial sections, and by comparison with cestodes of this genus from other avian hosts. Some confusion exists concerning the genera *Choanotaenia* Railliet, 1896, *Anomotaenia* Cohn, 1900, and *Paricterotaenia* Fuhrmann, 1932. The synonymy and relationships of these genera have been discussed by several writers (Johnston, 1911; Fuhrmann, 1932; Patwardhan, 1935; López-Neyra, 1935; Owen, 1946). A given cestode can often be assigned only with considerable difficulty to one of the above genera, since the character of the uterus cannot always be readily recognized. The writer has followed the arrangement of Joyeux and Baer (1936) for the European species of these genera. It is probable that some of the poorly described species will need to be reassigned to other genera, when additional material becomes available for study.

In addition to the few mammalian forms, there are at present about 50 apparently valid species of the genus *Choanotaenia* found in avian hosts. These cestodes are mainly parasites of charadriiform and passeriform birds. The mammalian species are not discussed in this paper, since they are not closely related to *C. speotytonis*.

A single species belonging to the genus under consideration has previously been recorded from strigiform hosts. *Choanotaenia strigium* Joyeux and Timon-David, 1934, was described from *Otus scops* (L.) in France. Prior to this record, all described owl cestodes belonged to the genus *Paruterina*. *Choanotaenia strigium* differs from the present species in having a larger number of testes (about 40), fewer hooks (12), an undivided ovary, and a different arrangement of the vitelline gland and seminal receptacle.

Owen (1946) described *C. numenii* from the long-billed curlew, and depended upon the position of the genital pore for differentiation from a number of other species. To the list of species with the genital pore in the anterior corner of the proglottid *C. speotytonis*, and no doubt others, can be added. It is often not possible to determine the position of the genital pore from the available description, and since the state of contraction of the strobila must also be taken into consideration when determining the relative position of the genital pore, it is the opinion of the writer that this character is of little diagnostic value.

Choanotaenia speotytonis can be distinguished apparently without difficulty from all other species of the genus which have been comparatively well

described. The main characters used for differentiation are considered here separately. It can be noted that most of the species differ from *C. speotytonis* in more than one main character.

Several species (*C. cayennensis* (Fuhrmann, 1907), *C. cayennensis africana* (Joyeux and Baer, 1928), *C. cingulifera* (Krabbe, 1869), *C. crateriformis* (Goeze, 1782), *C. dispar* Burt, 1940, *C. guiarti* (Tseng Chen, 1932), *C. iola* Lincicome, 1939, *C. macracantha* (Fuhrmann, 1907), *C. manipurensis* Patwardhen, 1935, *C. musculosa* (Fuhrmann, 1896), *C. passerina* (Fuhrmann, 1907), *C. secunda* (Fuhrmann, 1907), *C. sinensis* Joyeux and Baer, 1935, and *C. southwelli* (Fuhrmann, 1932) possess two rows of hooks, while *C. speotytonis* has only a single row. However, this character apparently cannot always be determined with certainty. *Choanotaenia chandleri* (Moghe, 1925) may have either 1 or 2 rows, and *C. meliphagidarum* Johnston, 1911, has been described as having an unarmed rostellum.

Several species differ from *C. speotytonis* in the number of hooks present on the rostellum; they are *C. abassenae* Joyeux, Baer, and Martin, 1936, *C. arctica* (Baylis, 1919), *C. burhini* Burt, 1940, *C. cayennensis* (Fuhrmann, 1907), *C. cayennensis africana* (Joyeux and Baer, 1928), *C. cayennensis scolopacis* Joyeux and Baer, 1939, *C. cingulifera* (Krabbe, 1869), *C. corvi* Joyeux, Baer, and Martin, 1936, *C. crateriformis* (Goeze, 1782), *C. delachauxi* (Baer, 1925), *C. delachauxi megacantha* López-Neyra, 1935, *C. dispar* Burt, 1940, *C. fieldingi* Maplestone and Southwell, 1923, *C. guiarti* (Tseng Chen, 1932), *C. glareolae* Burt, 1940, *C. gondwana* Inamdar, 1934, *C. infundibulum* (Bloch, 1779), *C. joyeuxi* Tseng Chen, 1932, *C. macracantha* (Fuhrmann, 1907), *C. magnihamata* Burt, 1940, *C. manipurensis* Patwardhan, 1935, *C. marchali* (Mola, 1907), *C. musculosa* (Fuhrmann, 1896), *C. numenii* Owen, 1946, *C. passerina* (Fuhrmann, 1907), *C. rostrata* (Fuhrmann, 1918), *C. secunda* (Fuhrmann, 1907), *C. sinensis* Joyeux and Baer, 1935, *C. southwelli* Fuhrmann, 1932, *C. stagnatildis* Burt, 1940, *C. stercoraria* (Baylis, 1919), *C. triganciencis* Joyeux and Baer, 1939, *C. tringae* Joyeux, Baer, and Martin, 1936, *C. uncinata* Fuhrmann, 1918, and *C. unicoronata* (Fuhrmann, 1908).

Although some species (*C. polyorchis* (Klaptocz, 1908), *C. microsoma* Southwell, 1922, *C. lobipluviae* Burt, 1940, and *C. iola* Lincicome, 1939) overlap in number of hooks present, they can be differentiated from *C. speotytonis* by other characters. The number of hooks is not known for *C. rostellata* (Fuhrmann, 1908), and *C. meliphagidarum* Johnston, 1911, has been described as having an unarmed rostellum. Both of these can be differentiated by other characters.

Hook measurements differ from those of *C. speotytonis* in a number of species: *C. abassenae* Joyeux, Baer, and Martin, 1936, *C. arctica* (Baylis, 1919), *C. burhini* Burt, 1940, *C. cayennensis africana* (Joyeux and Baer, 1928), *C. cayennensis scolopacis* Joyeux and Baer, 1939, *C. chandleri* (Moghe, 1925), *C. cingulifera* (Krabbe, 1869), *C. corvi* Joyeux, Baer, and Martin, 1936, *C. crateriformis* (Goeze, 1782), *C. delachauxi* (Baer, 1925), *C. delachauxi megacantha* López-Neyra, 1935, *C. dispar* Burt, 1940, *C. fieldingi* Maplestone and Southwell, 1923, *C. guiarti* (Tseng Chen, 1932), *C. glareo-*

lae Burt, 1940, *C. gondwana* Inamdar, 1934, *C. infundibulum* (Bloch, 1779), *C. iola* Lincicome, 1939, *C. joyeuxi* Tseng Chen, 1932, *C. lobipluviae* Burt, 1940, *C. macracantha* (Fuhrmann, 1907), *C. magnihamata* Burt, 1940, *C. manipurensis* Patwardhan, 1935, *C. microsoma* Southwell, 1922, *C. musculosa* (Fuhrmann, 1896), *C. numenii* Owen, 1946, *C. passerina* (Fuhrmann, 1907), *C. polyorchis* (Klaptocz, 1908), *C. rostrata* (Fuhrmann, 1918), *C. secunda* (Fuhrmann, 1907), *C. sinensis* Joyeux and Baer, 1935, *C. southwelli* Fuhrmann, 1932, *C. stagnatildis* Burt, 1940, *C. stercoraria* (Baylis, 1919), *C. triganciencis* Joyeux and Baer, 1939, *C. tringae* Joyeux, Baer, and Martin, 1936, and *C. uncinata* Fuhrmann, 1918.

Hook measurements overlap in only two cases, in *C. cayennensis* (Fuhrmann, 1907) and *C. unicoronata* (Fuhrmann, 1908); these are readily differentiated from *C. speotytonis* by means of other characters.

The number of testes differs from that of *C. speotytonia* in *C. abassenae* Joyeux, Baer, and Martin, 1936, *C. burhini* Burt, 1940, *C. cayennensis* (Fuhrmann, 1907), *C. cayennensis africana* (Joyeux and Baer, 1928), *C. chandleri* (Moghe, 1925), *C. cingulifera* (Krabbe, 1869), *C. corvi* Joyeux, Baer, and Martin, 1936, *C. delachauxi megacantha* López-Neyra, 1935, *C. glareolae* Burt, 1940, *C. infundibulum* (Bloch, 1779), *C. iola* Lincicome, 1939, *C. joyeuxi* Tseng Chen, 1932, *C. lobipluviae* Burt, 1940, *C. macracantha* (Fuhrmann, 1907), *C. magnihamata* Burt, 1940, *C. manipurensis* Patwardhan, 1935, *C. marchali* (Mola, 1907), *C. musculosa* (Fuhrmann, 1896), *C. numenii* Owen, 1946, *C. polyorchis* (Klaptocz, 1908), *C. rostellata* (Fuhrmann, 1908), *C. southwelli* Fuhrmann, 1932, *C. stagnatildis* Burt, 1940, *C. stercoraria* (Baylis, 1919), *C. tringae* Joyeux, Baer, and Martin, 1936, and *C. uncinata* Fuhrmann, 1918.

The number of testes overlaps that of *C. speotytonis* in *C. arctica* (Baylis, 1919), *C. chandleri* (Moghe, 1925), *C. crateriformis* (Goeze, 1782), *C. guiarti* (Tseng Chen, 1932), *C. delachauxi megacantha* López-Neyra, 1935, *C. dispar* Burt, 1940, *C. gondwana* Inamdar, 1934, *C. joyeuxi* Tseng Chen, 1932, *C. meliphagidarum* Johnston, 1911, *C. passerina* (Fuhrmann, 1907), *C. sinensis* Joyeux and Baer, 1935, and *C. unicoronata* (Fuhrmann, 1908). The number of testes is not known for *C. cayennensis* (Fuhrmann, 1907), *C. delachauxi* (Baer, 1925), *C. fieldingi* Maplestone and Southwell, 1923, *C. microsoma* Southwell, 1922, and *C. secunda* (Fuhrmann, 1907). These all differ in other characters from *C. speotytonis*.

Other differences occur, such as in length of cirrus sac, size of testes, and arrangement of the female reproductive organs; it is not necessary to discuss these in order to differentiate *C. speotytonis*.

Certain of the previously described species are not considered in this paper; the descriptions of *C. platycephala* (Rudolphi, 1809), *C. taylori* Johnston. 1912, and *C. zoniferae* Johnston, 1912, are not adequate. The descriptions of *C. missoevi* Skrjabin and Popoff, 1924, and *C. littorae* De Muro, 1934, could not be obtained.

ADDENDUM

After this paper was submitted for publication the writer had the opportunity to make certain observations on the life cycle of *Paruterina candelabraria* (Goeze, 1782).

A barred owl, *Strix v. varia* Barton, was collected on March 10, 1948, in central Wisconsin, and was found to be infected with two cestodes of this species. After the gravid proglottids were removed, the strobilae were fixed, stained, and mounted. Examination of the gravid proglottids revealed many eggs in the uteri and parauterine organs, and active embryos were observed within the eggs.

The gravid proglottids were fed to three young laboratory mice, *Mus musculus* L., and to a wild-trapped gray squirrel, *Sciurus carolinensis leucotis* Gapper. These animals were autopsied on the 29th and 30th days after the feeding of the proglottids. Cysticercoids were found in extremely large numbers in the livers of the mice, and the size and shape of the hooks showed these to be larvae of *Paruterina candelabraria*. No cysticercoids were observed in the squirrel.

Parts of the infected livers were fed to a great horned owl, *Bubo v. virginianus* (Gmelin), and it is hoped that there will be an opportunity to attempt to infect native North American mice. A complete report will be published elsewhere.

ACKNOWLEDGMENT

The writer wishes to express his indebtedness to Dr. Jean G. Baer, for the cestodes from European owls; to Dr. E. W. Price, for information from the files of the Zoological Division of the Bureau of Animal Industry; and to Mr. N. C. Negus, for supplying the burrowing owls used in this study.

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The Box Turtle as a Host for Dipterous Parasites

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The occurrence of myiasis in the box turtle, *Terrapene carolina,* although briefly noted by several authors, is almost completely undocumented in herpetological literature. The entomological literature is correspondingly scanty, and consists of a few very short notes, which merely report the observation of the infestation of a single specimen, with the exception of Aldrich's "Sarcophaga" (1916), and the detailed study by Knipling (1937).

All of the earlier authors found the larvae only in the neck or nuchal region of the turtle. Aldrich quotes an ambiguous label on his holotype, stating that the specimens were from "larva bred in sore in side of box turtle," which might be considered the first report of the larvae attacking elsewhere than in the neck region. Packard (1882) was the first to report the occurrence, and pictured the larvae. Wheeler (1890) first successfully bred the larvae, but he refused to describe the species as new, after assigning it to the genus *Sarcophaga.* Emerton (1904) also bred larvae, but here again no attempt was made to determine the species of *Sarcophaga* represented. Kepner (1912) gives an extensive report on the larva, but published his work before one pupating larva could hatch. He also assigned his specimens to *Sarcophaga,* with no species designation. Aldrich (*loc. cit.,* p. 278) described the species for the first time, assigning it the name *Sarcophaga cistudinis.* This is the most detailed account of the adult fly available. Emerton reported a total of 5 larvae, from a swelling on the left side of the neck; Kepner found 4 larvae, all on the right side of the neck; Packard quoted Prof. J. W. P. Jenks in stating that eight or ten were found under the skin of the back of the neck, close to the shell; True (1884) found 13 "bot-flies" in the muscles on either side of the neck; Wheeler found eight larvae in a swelling on the neck, near the carapace; Babcock (in letter) states that he found the larvae in a lump on the underside of the neck; Cahn (1937) found larvae in the neck region of two turtles. The localities in which the turtles were collected were Long Island, New York; Middleboro, Massachusetts; Cape Cod, Massachusetts; Plainfield, New Jersey; and Windsor, Connecticut. Kepner's specimen was probably taken in Virginia. Cahn gives no locality, it is presumably Illinois. Babcock (1919) and Pope (1939) both state that the infestation occurs, with no further discussion.

Townsend (1917) states that *Sarcophaga cistudinis* (for which he erected the new genus *Cistudinomyia*) was a "true parasite confined to the box-turtle." Knipling's report, however, was based primarily on specimens of *S. cistudinis* from *Gopherus polyphemus,* the gopher turtle. He mentions a single specimen of Terrapene from Florida in which he found larval flies, presumably of

the species *T. carolina,* but of a different subspecies. He suggests that the flies seek an abrasion or weakness in the skin of the turtle for a point of larviposition, and that the most often used weakness is scar-tissue left due to attacks by the tick, *Ambylomma tuberculatum* Marx. Larvae penetrated the skin of a tortoise through undamaged skin in one of eight experimental attempts. It is unfortunate that additional information on the records of D. G. Hall (briefly mentioned by Knipling) has not been published. It appears that he has seen more cases of turtle infestation (about 35) than are found in the entire literature on the subject. He found that 25% of all turtles collected over a three year period in Georgia were infested. The species of turtles are not given. McMullen (1940) also based his findings on another species, the ornate box turtle (*Terrapene ornata*), taken in Oklahoma. He gives the first report of infestation elsewhere than in the neck region in the genus Terrapene. Five lesions were found, the most anterior on the neck, the most posterior at the junction of the skin and the eleventh marginal scute. Three lesions were posterior to the shell bridge. He found a total of 27 larvae in the five lesions, with a range of 1 to 18. Chidester (1915) reported the larva from the painted turtle (*Chrysemys picta*).

Two specimens of *Terrapene carolina* collected by me in 1940 were found to be heavily attacked by this insect larvae. The first turtle was taken September 6, 1940, about 2½ miles west of Greenup, Cumberland County, Illinois, in a swampy section of bottom land, buried in the mud of a small pond. This turtle had a small hole through the flesh immediately anterior to the hind legs on either side, both of which were clogged with suppurative matter. After removing this matter, I took 15 larvae from the hole on the right side, and 2 from the left. On September 14, I removed additional matter from the left side, and two more larvae. The hole on the right side was healing rapidly, and was almost closed. Signs of suppurative matter were observed at the same time on the second specimen, collected on August 9, 1940, ½ mile west of Greenup, Cumberland County, Illinois, but this specimen was left undisturbed in the hope that some of the larvae would metamorphose. This had not been observed by November 20, so the infestations were cleaned out. The holes were in almost exactly the same position as in the first specimen, about three mm. anterior to the hind leg insertion. The hole on the left side was so full of larvae they protruded through it, and 14 were removed. Six larvae were taken from the hole on the right side. This specimen had lost all control of its hind legs, and they were dragged limply along in locomotion. This specimen died on November 28, and was dissected. A third hole was found above and just behind the right hind leg. This hole contained 2 larvae, the left lower side had 2, and the right lower side none. The total larvae in the first specimen was 19, in the second 24.

The larvae had attacked the lymph glands and the cloaca of the dissected specimen. The cloaca was enlarged and contained extraneous tissue, grown to accommodate the parasites. The lymph glands had also expanded their tissue, and had formed a thick wall about the space occupied by the larvae. The larvae affect muscular and nervous control, as is seen by the turtle's inability

to use its limbs, and were quite possibly the cause of death. The first specimen died on April 9, 1941, and was dissected at the time. The left side still showed a small hole, the right side was completely healed, and only a trace of scar tissue remained. Thus, the turtles are capable of surviving an attack by this parasite if the larvae are removed, although a heavy infestation may cause death.

Since no imagos were obtained, it cannot be safely ascertained that this is Aldrich's *Sarcophaga cistudinis*. If so, and I consider it probable, this constitutes a long extension of the known range, into central Illinois. It is interesting that this is the first time that the parasite has been found in the hind leg and anal region of this species of turtle, and that it is present in such numbers.

Six specimens of the larvae of this fly are now deposited in the collections of the University of Michigan, Museum of Zoology. Many thanks are due Dr. J. Speed Rogers, of this institution, for his kind assistance in the identification of the larvae.

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A List of Vascular Plants from St. Louis County, Minnesota

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In the summer of 1946, having an opportunity of spending a few weeks in northern Minnesota a short distance south of the International Boundary, the writer made a small collection of vascular plants available in that locality. While it is fairly obvious that the following list falls short of including all species of vascular plants to be found in that region, no doubt it contains most of the principal species, and will serve to give some idea of the flora at present growing there.

Other persons have made collections of plants in the general region although this is the first published list from this particular place. In August 1889, E. J. Hill* spent twelve days observing the flora and incidentally picking up a few plants near the town of Tower, and because his specimens are in the University of Illinois herbarium, they have been added to the list. Also, the collections of Professor W. V. Balduf of the University of Illinois have been included. In connection with his entomological work Professor Balduf has spent several summers in the region, and particularly during the summer of 1945 he made a collection of about one hundred species of vascular plants. He has not only turned over a set of these plants to the herbarium of the University of Illinois, but has kindly permitted the incorporation of the records of his collections into this list.

The following list includes 428 species, 242 genera, and 84 families of vascular plants. All specimens were collected within a radius of a dozen miles of Armstrong Lake, which is situated about half way between the towns of Ely and Tower in the northeastern part of St. Louis County. Specimens indicated by number only, without collector's name, are to be understood as having been collected by the writer, and unless otherwise indicated these came from the vicinity of Armstrong Lake. E. J. Hill's specimens, which came chiefly from Tower, and the south side of Lake Vermilion, are cited with the initials E. J. H., while those of Professor Balduf, mostly from Eaglenest Lake, are cited with the initials W. V. B. The first set of the writer's specimens is in the herbarium of the University of Illinois; other specimens have been distributed to several American herbaria.

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* In 1890, Hill published two short papers on his botanical observations, mentioning incidentally some twenty-three species of vascular plants observed or collected in the vicinity of Tower. These articles appeared in volume fifteen of the Botanical Gazette under the title: *Notes on the flora of the Lake Superior region, Minnesota*, pp. 304-311, and 324-331. For an account of the life and work of Rev. E. J. Hill see the excellent biographical sketch by Mrs. Agnes Chase in Rhodora 19: 61-69. 1917, which includes a portrait, and Hill's bibliography of 162 titles.

Division I. Pteridophyta—Ferns and Fern-allies

LYCOPODIACEAE Michx.—Clubmoss Family

Lycopodium annotinum L. Stiff Clubmoss. Eaglenest Lake, September 19, 1943, *W. V. B.*; woods, common, 18232.

Lycopodium clavatum L. Running-pine.—Woods, common, 18057, 18189.

Lycopodium lucidulum Michx. Shining Clubmoss.—Coniferous woods, Lake Vermilion, 18612.

Lycopodium obscurum L. Ground-pine.—Woods, 18238, 18640. Shady rich woods, Eaglenest Lake, *W. V. B.* in 1945.

Lycopodium tristachyum Pursh. Ground-pine.—Woods, 18187, 18357.

SELAGINELLACEAE Underw.—Selaginella Family

Selaginella rupestris (L.) Spring.—Dry exposed rocks, occasional, 18062.

ISOETACEAE Underw.—Quillwort Family

Isoetes braunii Dur. Braun's Quillwort.—Sandy bottom of Armstrong Lake, common, 18447, 18542.

Isoetes macrospora Dur. Lake Quillwort.—Sandy bottom of Armstrong Lake, in shallow water, 18551.

EQUISETACEAE Michx.—Horsetail Family

Equisetum arvense L. Field Horsetail.—Railroad embankments, roadsides, and sandy or gravelly shores, common, 18014, 18364. The latter number consists of branched leafy plants bearing a cone (var. *serotinum*). Only two such plants were seen.

Equisetum fluviatile L. Water Horsetail.—Shallow margins of lakes, or edges of bogs, common, 18090.

Equisetum hyemale L. Tall Scouring-rush.—Alluvial soil, occasional; many of last year's stems still standing; this year's stems cone-bearing toward the end of July, 18065, 18339, 18398, 18498, 18654.

Equisetum sylvaticum L. Wood Horsetail.—Abundant in moist ground, 18188; edge of woods near Eaglenest Lake, July 12, 1945, *W. V. B.*

OPHIOGLOSSACEAE Presl—Adder's-tongue Family

Botrychium mutifidum (S. G. Gmel.) Rupr. Leathery Grapefern.—Grassy hillside, rare, 18710.

Botrychium virginianum (L.) Sw. Rattlesnake Fern.—Grassy open woods, not uncommon, 18272.

OSMUNDACEAE R. Br.—Royal Fern Family

Osmunda cinnamomea L. Cinnamon Fern.—Swampy ground, common, 18350.

Osmunda claytoniana L. Interrupted Fern.—Boggy meadows and borders of thickets, rather common, 18353, 18613.

POLYPODIACEAE R. Br.—Fern Family

Athyrium angustum (Willd.) Presl. Lady Fern.—Abundant in swampy ground in woods and meadows, 18604, 18644.

Cystopteris fragilis (L.) Bernh. Brittle Fern.—In clefts of rocks, not common, 18415.

Dryopteris cristata (L.) A. Gray. Crested Wood Fern.—Swampy woods, common, 18116, 18196.

Dryopteris disjuncta (Ledeb.) Morton. Oak Fern.—Woods, occasional, 18058; Lake Vermilion, 18614.

Dryopteris phegopteris (L.) C. Chr. Long Beech Fern.—Woods, 18185.

Dryopteris spinulosa (O. F. Muell.) Watt. Spinulose Wood Fern.—Woods, common, 18055, 18114, 18147.

Dryopteris thelypteris (L.) A. Gray. Marsh Fern.—Marshy ground, common, 18342.

Onoclea sensibilis L. Sensitive Fern.—Moist woods or edges of meadows, occasional, 18215, 18747.

Polypodium virginianum L. Common Polypody.—Clefts of rocks, common, 18184.

Pteretis struthiopteris (L.) Nieuwl. Ostrich Fern.—Moist thickets, rare, 18229, 18556.

Pteridium latiusculum (Desv.) Hieron. Bracken.—Common everywhere in open woods, 18115, 18733.

Woodsia obtusa (L.) R. Br. Rusty Cliff Fern.—Clefts of rocks, infrequent, 18253, 18416.

Woodsia oregana D. C. Eaton. Oregon Woodsia.—Crevices of exposed rocks, rare, 18091.

Division II. Spermatophyta—Seed Plants

Subdivision I. Gymnospermae—Gymnosperms

Taxaceae Lindl.—Yew Family

Taxus canadensis Marsh. Canada Yew.—Wooded ravine, Ely, rare, 18637.

Pinaceae Lindl.—Pine Family

Abies balsamea (L.) Mill. Balsam Fir.—Woods, common, 18107, 18645.

Larix laricina (DuRoi) K. Koch. Tamarack.—Chiefly in bogs and swampy woods, common, 18118.

Picea mariana (Mill.) BSP. Black Spruce.—Usually in bogs, with *Larix*, or in swampy ground, common, 18167, 18352.

Pinus banksiana Lamb. Jack Pine.—Rocky acid soils, common, 18342, 18588; Eaglenest Lake, September 19, 1945, *W. V. B.*

Pinus resinosa Ait. Red Pine.—Chiefly in sandy or rocky soil, often along lake shores, 18419.

Pinus strobus L. White Pine.—Scattered throughout the region, 18589.

Cupressaceae Neger—Cypress Family

Juniperus canadensis Burgsd. Low Juniper.—Rocky woods, rare, Ely, 18626.

Thuja occidentalis L. Arbor-vitae.—In bogs, or on rocky slopes, common, 18561; Lake Vermilion, 18607.

Subdivision II. Angiospermae—Flowering Plants

Class 1. Monocotyledoneae Juss.

Typhaceae J. St. Hil.—Cat-tail Family

Typha latifolia L. Common Cat-tail.—Swamps or ditches, very common, 18299; margin of Eaglenest Lake, September 1, 1945, *W. V. B.*

Sparganiaceae Agardh.—Bur-reed Family

Sparganium americanum Nutt.—In slow streams and edges of ponds and lakes, common, 18732.

Sparganium angustifolium Michx.—West Two Rivers, near Tower, August 26, 1889, *E. J. H.*

Naiadaceae Lindl.—Naiad Family

Naias flexilis (Willd.) R. & S.—Common in shallow water, 18452, 18668; Eaglenest Lake, August 30, 1945, *W. V. B.*

POTAMOGETONACEAE Engler.—Pondweed Family

Potamogeton alpinus Balbis.—West Two Rivers, August 26, 1889, *E. J. H.*

Potamogeton amplifolius Tuckerm. Broad-leaved Pondweed.—In lakes, common, 18418, 18440, 18540, 18548; Eaglenest Lake, August 3, 1945, *W. V. B.*; East Two Rivers, August 17, 1889, *E. J. H.*

Potamogeton epihydrus Raf.—West Two Rivers, Tower, August 26, 1889, *E. J. H.*

Potamogeton gramineus L.—Shallow water of lakes, common, 18075, 18258, 18426, 18429, 18535, 18538, 18547, 18663; Lake Vermilion, August 23, 1889, *E. J. H.*

Potamogeton friesii Rupr.—East Two Rivers, August 27, 1889, *E. J. H.*; Tower, August 21, 1889, *E. J. H.*

Potamogeton natans L. Common Pondweed.—Eaglenest Lake, 18560; margin of Eaglenest Lake, August 30, 1945, *W. V. B.*

Potamogeton obtusifolius Mert. & Koch.—West Two Rivers, Tower, August 26, 1889, *E. J. H.*

Potamogeton praelongus Wulfen.—Shallow water, Eaglenest Lake, August 29, 1945, *W. V. B.*

Potamogeton pusillus L.—Lakes, common, 18543. Pools, Tower, August 21, 1889, *E. J. H.*

Potamogeton richardsonii (Benn.) Rydb.—Shallow water of lakes, common, 18077, 18465.

Potamogeton robbinsii Oakes.—In five feet of water, Eaglenest Lake, August 30, 1945, *W. V. B.*

Potamogeton spirillus Tuckerm.—Lakes, common, 18666.

Potamogeton zosteriformis Fern.—In five feet of water, Eaglenest Lake, August 30, 1945, *W. V. B.*

ALISMACEAE DC.—Water-plantain Family

Alisma subcordatum Raf. Water-plantain.—Ditches, ponds, or in cat-tail swamps, occasional, 18564.

Sagittaria cuneata Sheld.—In shallow water, infrequent, 18332. Tower, 18623; West Two Rivers, August 26, 1889, *E. J. H.*

Sagittaria latifolia Willd.—Wet ground along stream, 18667, 18685.

HYDROCHARITACEAE Aschers.—Frogbit Family

Anacharis canadensis (Michx.) Planch. Canadian Waterweed.—In five feet of water, Eaglenest Lake, August 30, 1945, *W. V. B.*

Vallisneria americana Michx. Eelgrass.—Shallow lakes, common, 18544.

GRAMINEAE Juss.—Grass Family

Agropyron repens (L.) Beauv. Quack Grass.—A common weed of waste places and roadsides, 18327.

Agropyron subsecundum (Link) Hitchc.—Eaglenest Lake, August 26, 1945, *W. V. B.*

Agrostis scabra Willd. Tickle Grass.—Common in sandy soil, 18331.

Alopecurus aequalis Sobol. Foxtail.—Wet ground, Tower, 18624.

Andropogon furcatus Muhl.—Grassy hillside, 18704.

Avena sativa L. Oat.—Occasionally spontaneous in waste ground, 18459.

Bromus ciliatus L.—Hillsides and open woods, common, 18474.

Bromus inermis Leyss. Hungarian Brome Grass.—Along roads, occasional, 18321, 18500.

Bromus kalmii A. Gray.—Dry hillsides, occasional, 18261.

Calamagrostis canadensis (Michx.) Beauv. Blue-joint Grass.—Swamps and low ground, common, 18112; Eaglenest Lake, August 26, 1945, *W. V. B.*

Cinna latifolia (Trev.) Griseb. Drooping Woodreed.—Damp woods by stream, Tower, August 17,1889, *E. J. H.*; wet ground in woods, Ely, 18636.

Danthonia spicata (L.) Beauv. Oat Grass.—Thin gravelly soil in open woods, 18642.

Digitaria ischaemum (Schreb.) Muhl.—Waste ground, 18711.

Elymus canadensis L. Nodding Wild Rye.—Sandy soil along railroad, occasional, 18393.

Elymus villosus Muhl.—Open woods, 18558.

Eragrostis pectinacea (Michx.) Nees.—In sandy soil, 18756.

Festuca ovina L. Sheep Fescue.—Sandy or gravelly soil, common, 18375.

Glyceria canadensis (Michx.) Trin.—Grassy roadsides, infrequent, 18470, wet ground, 18689.

Glyceria fernaldii St. John.—Swampy ground in woods, rare, 18148.

Glyceria grandis S. Wats. — Wet ground, Eaglenest Lake, August 29, 1945, *W. V. B.*; swamps, 18301.

Hordeum jubatum L. Squirrel-tail Grass.—Roadsides, not very common, 18330.

Muhlenbergia foliosa (R. & S.) Trin.—Meadow, 18649, 18768.

Muhlenbergia racemosa (Michx.) BSP.—Eaglenest Lake, August 26, 1945, *W. V. B.*

Oryzopsis asperifolia Michx.—Rocky open woods, common, 18643.

Panicum capillare L. Witch Grass.—Sandy soil along railroad, 18328, 18591.

Panicum latifolium L.—Rocky woods, not common, 18099.

Panicum tennesseense Ashe.—Moist ground, infrequent, 18063, 18344.

Phalaris arundinacea L. Canary Grass.—Edges of swamps, occasional, 18399, 18438.

Phleum pratense L. Timothy.—Weed of roadsides and waste places, common, 18323.

Phragmites communis Trin. Common Reed.—Margins of lakes, occasional, 18601; in shallow water, rocky margin of Eaglenest Lake, September 2, 1945, *W. V. B.*

Poa alsodes A. Gray.—Woods, 18369.

Poa annua L. Annual Blue Grass.—Moist ground, occasional, 18503.

Poa compressa L. Canada Blue Grass.—Waste places, common, 18381. A common grass in this locality, largely replacing *P. pratensis* that is so abundant farther south.

Poa pratensis L. Kentucky Blue Grass.—Roadsides and waste places, 18559.

Setaria viridis (L.) Beauv. Green Foxtail.—Weed in cultivated ground and in waste places, occasional, Ely, 18592.

Sporobolus cryptandrus (Torr.) A. Gray.—Sandy soil, 18712.

CYPERACEAE J. St. Hil.—Sedge Family

Carex aenea Fern.—Open woods, 18176.

Carex brunnescens Poir.—Bogs in woods, 18111.

Carex gracillima Schw.—Moist woods, 18347.

Carex grayii Carey.—Woods, local, 18182.

Carex lanuginosa Michx.—Moist ground, common, 18080.

Carex leptalea Wahlenb.—Bogs, 18348.

Carex pauciflora Lightf.—Sphagnum bog, 18164.

Carex paupercula Michx.—Sphagnum bog, 18092.

Carex pseudocyperus L.—Swamp, 18305, 18300.

Carex retrorsa Schk.—Swampy ground, 18195, 18304, 18358; wet ground near Tower, 18622.

Carex rosea Schk.—Woods, 18349.

Carex scoparia Schk.—Lake shore, Eaglenest Lake, July 11, 1945, *W. V. B.*; gravelly shores, common, 18051, 18371, woods, Ely, 18634.

Carex stipata Muhl.—Wet meadows, 18297, 18359.

Carex stricta Lam.—Wet meadows, 18193.

Carex trisperma Dewey.—Wet ground in woods, 18345.

Carex vesicaria L.—Wet meadows, and swamps, 18407.

Eleocharis acicularis (L.) R. & S.—Gravelly lake shore, 18370.

Eleocharis obtusa (Willd.) Schult.—Moist sandy soil, infrequent, 18471.

Eleocharis palustris (L.) R. & S.—Shallow water, common, 18411.

Eriophorum tenellum Nutt.—Bogs, common, 18093.

Scirpus acutus Muhl.—Shallow water, Eaglenest Lake, August 6, 1945.

Scirpus atrocinctus Fern.—Railroad ditch, Eaglenest Lake, July 12, 1945, *W. V. B.*

Scirpus cyperinus (L.) Kunth.—Eaglenest Lake, September 19, 1943, *W. V. B.*; wet meadows, abundant, 18006, 18472, 18690.

Scirpus validus Vahl.—Shallow water, margins of lakes, common. Stems 2-3 m. tall, 18422, 18427, 18670.

ARACEAE Necker—Arum Family

Acorus calamus L. Sweet Flag.—Wet ground along streams, common, 18688.

Calla palustris L. Water Arum.—In shallow water in swampy areas, not uncommon, 18764.

LEMNACEAE Dumort.—Duckweed Family

Lemma minor L.—Locally abundant on stagnant water, 18086.

JUNCACEAE Vent.—Rush Family

Juncus articulatus L. Jointed Rush.—Sandy shores, occasional, 18750.

Juncus bufonius L.—Sandy soil, occasional, 18469.

Juncus effusus L. Common Rush.—Moist ground, forming tufts or clumps, 18198, 18744.

Juncus pelocarpus E. Mey. Brown-fruited Rush.—Sandy shores, occasional, 18533.

Juncus tenuis Willd. Path Rush.—Sandy soil, often in roadways and along paths, common; observed but not collected.

Luzula saltuensis Fern.—Rocky open woods, rare, 18018.

LILIACEAE Adans.—Lily Family

Clintonia borealis (Ait.) Raf.—Woods near Ely, September 4, 1944, *W. V. B.*; coniferous woods, common, 18385.

Maianthemum canadense Desf.—Ely, August 28, 1944, *W. V. B.*; coniferous woods, common, 18240, 18435.

Streptopus roseus Michx. Twisted-stalk.—Woods, rare, 18384; Ely, 18632.

Uvularia sessilifolia L. Bellwort.—Woods, rare, 18255.

IRIDACEAE Lindl.—Iris Family

Iris versicolor L.—Wet ground, Eaglenest Lake, July 9, 1945, *W. V. B.*; swamps and wet meadows, common, 18066, 18687.

Sisyrinchium campestre Bickn.—Grassy hillside, not common, 18291.

ORCHIDACEAE Lindl.—Orchid Family

Corallorhiza maculata Raf. Spotted Coralroot.—Coniferous woods, occasional, 18201, 18314, 18705.

Habenaria huronensis (Nutt.) Spreng.—Wet meadows, common, 18202.

Malaxis unifolia Michx. Adder's-mouth Orchid.—Dry rocky woods, rare, 18256.

Spiranthes gracilis (Bigel.) Beck.—In grass on rocky ridge, Eaglenest Lake, July 27, 1945, *W. V. B.*; wooded hills, Tower, August 22, 1889, *E. J. H.*; grassy bank in open woods, rare, 18510.

Class II. DICOTYLEDONEAE Juss.

SALICACEAE Lindl.—Willow Family

Populus grandidentata Michx. Large-toothed Aspen.—Gravelly soil not uncommon, 18044, 18590.

Populus tacamahaca Mill. Balsam Poplar.—Moist ground, abundant, 18026.

Populus tremuloides Michx. Quaking Aspen.—Very abundant throughout the region, 18728.

Salix bebbiana Sarg. Bebb Willow.—Common in roadside ditches, Ely, July 9, 1945, *W. V. B.*; moist ground, one of the common willows of the region, 18121, 18252, 18378.

Salix discolor Muhl. Pussy Willow.—Common in moist ground, 18073, 18145, 18401.

Salix lucida Muhl. Shining Willow.—Gravelly shores, common and conspicuous, 18070, 18157, 18310.

Salix pedicellaris Pursh. Bog Willow.—Bogs, 18096.

Salix petiolaris Sm. Slender Willow.—Swampy ground, common, 18119, 18142, 18278, 18400, 18499.

Salix pyrifolia Anders. Balsam Willow.—Swamps, occasional, 18117, 18731.

Salix vitellina L. Golden Willow.—Wet ground near road, escaped from cultivation, 18179.

MYRICACEAE Dum.—Bayberry Family

Comptonia peregrina (L.) Coult. Sweetfern.—Rocky ridge, Eaglenest Lake, September 7, 1946, *W. V. B.*; gravelly soil in open woods, occasional, 18005.

Myrica gale L. Bog-myrtle.—In swamps and along lakes, abundant, 18022, 18437.

BETULACEAE Agardh.—Birch Family

Alnus crispa (Ait.) Pursh. Green Alder.—Shores and wooded slopes, common, 18023, 18102.

Alnus rugosa (DuRoi) Spreng. Speckled Alder.—(*A. incana* Am. auth.) Moist ground, very common, 18201.

Betula glandulifera (Regel) Butler. Dwarf Birch.—Swampy ground in woods, occasional, 18087, 18648.

Betula lutea Michx. f. Yellow Birch.—Moist wooded ravine, near Ely, rare, 18625.

Betula papyrifera Marsh. Paper Birch.—Very abundant; the characteristic deciduous tree of the region, 18033, 18141, 18143, 18268, 18522, 18578, 18646.

Corylus cornuta Marsh. Beaked Hazel.—Thickets and open woods, common, 18050, 18266.

FAGACEAE A. Br.—Beech Family

Quercus rubra L. Red Oak.—Rocky woods, rare, 18068.

Quercus macrocarpa Michx. Bur Oak.—Woods, rare, 18403.

ULMACEAE Mirb.—Elm Family

Ulmus americana L. American Elm.—Alluvial soil, not common, 18214.

URTICACEAE Reichenb.—Nettle Family

Urtica procera Muhl. Common Nettle.—Moist ground, locally abundant, alluvial soil, Ely, 18570.

SANTALACEAE R. Br.—Sandalwood Family

Comandra umbellata (L.) Nutt.—Rocky open woods, occasional, 18095, 18333.

POLYGONACEAE Lindl.—Buckwheat Family

Polygonum aviculare L. Knotweed.—A common weed in gravelly or sandy soil, 18183, 18652.

Polygonum cilinode Michx.—Roadside, Eaglenest Lake, July 8, 1945, *W. V. B.*; moist thickets and edges of woods, common, 18083, 18178, 18335.

Polygonum lapathifolium L.—Lake shore, Eaglenest Lake, August 9, 1945, *W. V. B.*; gravelly shores, common, 18126, 18158, 18553, 18577.

Polygonum natans (Michx.) Eaton.—Eaglenest Lake, September 2, 1945, *W. V. B.*; shallow water, common, 18432, 18545.

Polygonum natans forma *hartwrightii* (A. Gray) Stanford.—Swampy ground in moist thicket near lake, 18324.

Polygonum punctatum Ell.—Lake shores, occasional, 18528.

Rumex acetosella L. Field Sorrel.—Along railroad near Eaglenest Lake, August 9, 1945, *W. V. B.*; sandy soil, common, 18325.

Rumex crispus L. Curly Dock.—Waste ground and roadsides, common, 18482.

Rumex occidentalis Wats.—Moist meadows, common, 18069, 18308, 18494.

Rumex triangulivalvis (Danser) Rech. f. (*R. mexicanus* sensu auth.).—Gravelly shores and roadsides, 18021, 18458, 18517.

CHENOPODIACEAE Dumort.— Goosefoot Family

Chenopodium album L. Lamb's Quarter.—Roadsides and waste places, not common, 18388, 18696.

Chenopodium hybridum L. Maple-leaved Goosefoot.—Waste ground, occasional, 18313.

Salsola pestifer A. Nels. Russian Thistle.—Sandy soil along railroads, occasional, 18745.

AMARANTHACEAE J. St. Hil.—Amaranth Family

Amaranthus graecizans L. Tumbleweed.—Waste ground along railroad, occasional, 18721, 18770.

Amaranthus retroflexus L. Rough Pigweed.—Waste places, not common, Elv. 18572.

AIZOACEAE A. Br.—Carpetweed Family

Mollugo verticillata L. Carpetweed.—Gravel bank along railroad, Eaglenest Lake, August 9, 1945, *W. V. B.*; sandy soil, local, 18338, 18741.

PORTULACACEAE Reichenb.—Purslane Family

Portulaca oleracea L. Purslane.—Waste ground, occasional, 18681.

CARYOPHYLLACEAE Reichenb.—Pink Family

Cerastium vulgatum L. Common Mouse-ear Chickweed.—Eaglenest Lake, August 9, 1945, *W. V. B.*; a common weed in sandy or gravelly soil, 18172.

Lychnis alba Mill. Evening Campion.—Waste places, occasional, 18508.

Saponaria officinalis L. Bouncing Bet. Observed in Tower.

Silene antirrhina L. Sleepy Catchfly.—Gravelly soil, common, 18234.

Silene cucubalus Wibel. Bladder Catchfly.—Roadsides, Eaglenest Lake, July 11, 1945, *W. V. B.*; an occasional roadside weed, 18007.

Stellaria borealis Bigel.—Gravelly shores, occasional, 18191.

Stellaria longifolia Muhl. Long-leaved Chickweed.—Wet meadows, 18233.

Stellaria media (L.) Vill. Common Chickweed.—An occasional weed in cultivated ground, 18495.

RANUNCULACEAE Juss.—Buttercup Family

Actaea rubra (Ait.) Willd. Red Baneberry.—Woods, Eaglenest Lake, August 25, 1945, *W. V. B.;* woods not infrequent, 18226.

Actaea rubra f. *neglecta* (Gillman) Robins.—Eaglenest Lake, *W. V. B.;* open woods, occasional, 18567. Berries ivory-white, glossy.

Anemone canadensis L. Meadow Anemone.—Eaglenest Lake, July 9, 1945, *W. V. B.;* meadows or open woods, local, Tower, 18619.

Anemone cylindrica A. Gray.—Gravelly soil in open woods, common, 18108, 18206, 18282, 18363, 18394, 18480.

Aquilegia canadensis L. Columbine.—Dry rocky woods, occasional, Eaglenest Lake, July 13, 1945, *W. V. B.;* gravelly soil in open woods, common, 18693.

Caltha natans Pall.—Still or slowly flowing water; stems 1-2 feet long, rooting in the mud, Tower, August 21, 1889, *E. J. H.;* pools and streams, Tower, August 2, 1889, *E. J. H.;* Lake Vermilion, brooks and muddy shores June 1891, *J. H. Sandberg.*

Caltha palustris L. Marsh-marigold.—Swamps and bogs, common, 18665.

Clematis verticillaris DC. Purple Virgin's Bower.—Rocky woods, climbing over bushes, infrequent, 18413.

Ranunculus acris L. Tall Buttercup.—Eaglenest Lake, July 8, 1945, *W. V. B.;* roadsides and meadows, common and conspicuous, 18009.

Ranunculus flabellaris Raf.—On mud or in shallow water, occasional, 18298.

Ranunculus pennsylvanicus L. f.—In wet soil, Eaglenest Lake, July 28, 1945, *W. V. B.;* moist ground, common, 18059, 18526, 18775.

Ranunculus purshii Richards.—Muddy ground, Tower, August 17, 1889, *E. J. H.;* on mud or in shallow water, rare, 18298.

Ranunculus reptans L. Creeping Buttercup.—Gravelly shores, 18032.

Ranunculus trichophyllus Chaix.—Slow stream, Tower, August 17, 1889, *E. J. H.*

Thalictrum dasycarpum Fisch. & Lall.—Purplish Meadow-rue. Meadows, 18205, 18316, 18406; Tower, 18617.

NYMPHAEACEAE DC.—Waterlily Family

Nuphar variegatum Engelm. Yellow Pond-lily.—Common in shallow water in lakes, 18541, 18677, 18706.

Nymphaea odorata Ait. Fragrant Waterlily.—Eaglenest Lake, August 30, 1945, *W. V. B.;* shallow water in lakes, frequent, 18446, 18584.

FUMARIACEAE DC.—Fumitory Family

Corydalis aurea Willd. Golden Corydalis.—Along railroad, Eaglenest Lake, July 21, 1945, *W. V. B.*

Corydalis sempervirens (L.) Pers. Pink Corydalis.—Rocky open woods; plants very glaucous, 18054, 18250.

CRUCIFERAE B. Juss.—Mustard Family

Berteroa incana (L.) DC. Hoary Alyssum.—Roadside weed, near Tower, 18067.

Brassica nigra (L.) Koch. Black Mustard.—Waste places, occasional, 18443.

Capsella bursa-pastoris (L.) Medic. Shepherd's Purse.—A weed in waste places, 18683.

Cardamine pennsylvanica Muhl.—In moist ground or along gravelly shores, 18026.

Erysimum cheiranthoides L. Wormseed Mustard.—Along railroad, Eaglenest Lake, July 11, 1945, *W. V. B.*

Erysimum inconspicuum (S. Wats.) MacM.—Dry gravelly or sandy soil, occasional, 18035.

Lepidium densiflorum Schrad. Common Peppercress.—Roadsides, not very common, 18160, 18380.

Rorippa hispida (DC.) Britt.—Wet ground in woods, not common, 18365.

Rorippa islandica (Oeder) Borbás. Yellow Cress.—Moist ground, frequent, 18004.

Sisymbrium altissimum L. Tumble Mustard.—Waste places, infrequent, 18177.

SARRACENIACEAE LaPyl.—Pitcher-plant Family

Sarracenia purpurea L. Pitcher-plant.—Sphagnum bogs, 18386.

DROSERACEAE S. F. Gray—Sundew Family

Drosera rotundifolia L. Round-leaved Sundew.—Sphagnum bogs, common, 18782.

PARNASSIACEAE Dum.—Grass-of-Parnassus Family

Parnassia palustris L. Northern Grass-of-Parnassus.—On mossy log in stream, rare, 18180.

SAXIFRAGACEAE Dum.—Saxifrage Family

Heuchera richardsonii R. Br. Alumroot.—Rocky slopes, locally abundant, 18010.

GROSSULARIACEAE Dum.—Gooseberry Family

Ribes americanum Mill. American Black Currant.—Moist thickets, 18280, 18309.

Ribes glandulosum Weber. Skunk Currant.—Moist rocky woods, 18017, 18103, 18224.

Ribes hirtellum Michx. Wild Gooseberry.—Rocky woods, 18072, 18101, 18225.

Ribes triste Pall. Swamp Red Currant.—Moist rocky woods, 18017.

ROSACEAE Juss.—Rose Family

Agrimonia striata Michx.—Thickets, meadows, and open woods, common, 18231, 18336, 18376, 18505, 18530.

Amelanchier bartramiana (Tausch) M. Roem.—Vermilion Lake, *Arthur, Bailey & Holway*, B407.

Amelanchier interior Nielsen.—Rocky woods, occasional, 18361, 18484.

Amelanchier laevis Wieg. Smooth Juneberry.—Woods, common, 18175, 18267.

Amelanchier sanguinea (Pursh.) DC. Shadbush.—Open woods, the common shadbush of the region, 18043, 18211, 18270, 18377, 18397, 18410, 18493.

Fragaria americana (Porter) Britt.—Woods, common, 18003.

Fragaria virginiana Duch. Wild Strawberry.—Open woods, common, 18013, 18199; gravelly lake shore, 18682.

Geum macrophyllum Willd. Large-leaved Avens.—Wet ground, Tower, August 21, 1889, *E. J. H.*

Geum rivale L.—Wet meadows, occasional, 18307.

Geum strictum Ait. Yellow Avens.—Roadside, Eaglenest Lake, August 26, 1945, *W. V. B.*; meadows and open woods ,common, 18011, 18146, 18320.

Potentilla arguta Pursh. Tall Cinquefoil.—Open grassy places, Eaglenest Lake, July 13, 1945, *W. V. B.*; rocky slopes, local, 18283.

Potentilla monspeliensis L. Rough Cinquefoil.—Along railroad, July 11, 1945, *W. V. B.*; roadsides, very common, 18213, 18462.

Potentilla palustris (L.) Scop. Purple Cinquefoil.—Wet ground, Eaglenest Lake, July 9, 1945, *W. V. B.*; bogs, not infrequent, 18192.

Potentilla tridentata Ait. Three-toothed Cinquefoil.—Rocky coniferous woods, rare in this region, 18081.

Prunus pennsylvanica L. f. Wild Red Cherry.—Abundant in cutover land, 18025.

Prunus virginiana L. Chokecherry.—Common in open woods, 18263, 18444, 18566.

Rosa acicularis Lindl.—Rocky or gravelly soil in open woods, or at edges of thickets; apparently less common in this locality than *R. blanda*, 18431, 18442.

Rosa blanda Ait. Meadow Rose. (*R. engelmanni* sensu E. J. Hill.).—Low rich ground, Tower, August 21, 1889, *E. J. H.*; Eaglenest Lake, September 19, 1943, *W. V. B.*; 18037, 18154, 18207, 18230, 18279, 18389, 18445, 18563, 18608, 18629.

Rubus argutus Link. Tall Blackberry.—Open woods, common, 18046.

Rubus flagellaris Willd. Moist woods, 18064.

Rubus pubescens Raf. Dwarf Raspberry.—Occasional at edge of woods, Eaglenest Lake, July 22, 1945, *W. V. B.*; woods, 18123.

Rubus strigosus Michx. Wild Red Raspberry.—Open woods, common, 18028. 18144, 18599.

Sorbus americana Marsh. American Mountain-ash.—Woods, common, 18056, 18476.

Sorbus decora (Sarg.) Schneid.—Woods, common, 18089, 18248, 18513.

Spiraea alba DuRoi.—Lake shore, Eaglenest Lake, July 28, 1945, *W. V. B.*; swamps and wet meadows, common, 18396, 18277; Lake Vermilion, 18610.

LEGUMINOSAE Juss.—Pea Family

Amorpha canescens Pursh. Lead Plant.—Sandy soil in open woods, 18596, 18675.

Astragalus canadensis L. Milk-vetch.—Gravelly soil, Ely, 18636.

Lathyrus ochroleucus Hook.—Edge of woods, Eaglenest Lake, July 12, 1945, *W. V. B.*; near Tower, August 21, 1889, *E. J. H.*; thickets and open woods, common, 18036, 18285.

Lathyrus venosus Muhl.—Near Tower, August 21, 1889, *E. J. H.*; thickets and open woods, common, 18047.

Medicago lupulina L. Black Medic.—Roadside weed, occasional, 18694.

Medicago sativa L. Alfalfa.—Roadsides, occasional, Tower, 18616.

Melilotus alba Desr. White Sweet Clover.—Roadsides, occasional, 18269, 18502.

Melilotus officinalis (L.) Lam. Yellow Sweet Clover.—Roadsides, occasional, 18501.

Petalostemum purpureum (Vent.) Rydb. Purple Prairie-clover.—Gravelly soil, Ely, 18635.

Trifolium hybridum L. Alsike Clover.—Roadsides and waste places, common, 18730.

Trifolium pratense L. Red Clover.—Roadsides and waste places, common, 18265.

Trifolium procumbens L. Low Hop-clover.—Gravelly roadside, occasional, 18181.

Trifolium repens L. White Clover.—Roadsides and waste places, common.

Vicia americana Muhl. American Vetch.—Railroad bank, Ely, September 23, 1944, *W. V. B.*; near Tower, August 21, 1889, *E. J. H.*; thickets and open woods, common, 18015.

GERANIACEAE J. St. Hil.—Geranium Family

Geranium bicknellii Britt.—Along railroad, Eaglenest Lake, July 13, 1945, *W. V. B.*; burned places in woods near dwellings, 18246.

BALSAMINACEAE Lindl.—Jewel-weed Family

Impatiens biflora Walt. Touch-me-not.—In moist places, Eaglenest Lake, August 24, 1945, *W. V. B.*; wet shaded ground, Tower, August 23, 1889, *E. J. H.*; wet ground, common, 18156, 18576, 18661, 18774.

EUPHORBIACEAE J. St. Hil.—Spurge Family

Chamaesyce glyptosperma (Engelm.) Small.—Sandy soil along railroad, 18341, 18680, 18752.

Euphorbia corollata L.—Gravelly soil on railroad embankment, not common, 18204.

CALLITRICHACEAE Lindl.—Water-starwort Family

Callitriche palustris L.—In a slow stream, 18241.

AQUIFOLIACEAE DC.—Holly Family

Ilex verticillata (L.) A. Gray. Winterberry.—Eaglenest Lake, September 17, 1945, *W. V. B.;* wet ground in thicket near lake, 18518.

ANACARDIACEAE Lindl.—Sumac Family

Rhus glabra L. Smooth Sumac.—Eaglenest Lake, September 19, 1943, *W. V. B.;* common on hillsides and in open woods, 18486, 18515.

Rhus radicans L. Poison-ivy.—Rocky woods and thickets, not uncommon. Low shrubs, 30-60 cm. tall, 18519.

ACERACEAE Lindl.—Maple Family

Acer rubrum L. Red Maple.—Rocky woods, common, 18264.

Acer spicatum Lam. Mountain Maple.—Abundant throughout the region, 18049, 18106, 18492.

RHAMNACEAE Dum.—Buckthorn Family

Rhamnus alnifolia L'Her. Alder Buckthorn.—Swamps and edges of woods, 18449.

VITACEAE Lindl.—Grape Family

Parthenocissus vitacea (Kern.) K. Fritsch. Woodbine.—Trailing or low-climbing, woods, common, 18227.

TILIACEAE Juss.—Linden Family

Tilia americana L. Basswood.—Moist woods, common, 18222.

HYPERICACEAE Lindl.—St. John's-wort Family

Triadenum virginicum (L.) Raf. Marsh St. John's-wort.—In moist grassy places, Eaglenest Lake, July 28, 1945, *W. V. B.;* swamps and bogs, and edges of moist thickets, 18149, 18586, 18743.

CISTACEAE Lindl.—Rockrose Family

Lechea intermedia Leggett. Pinweed.—Dry hills, under *Pinus banksiana*, near Tower, August 21, 1889, *E. J. H.;* also Jasper Peak, August 24, 1889, *E. J. H.*

VIOLACEAE DC.—Violet Family

Viola canadensis L. Canada Violet.—Woods, 18217.

Viola palustris L. In woods, Eaglenest Lake, in 1945, *W. V. B.;* wet ground in woods, 18236.

Viola subvestita Greene.—Sandy soil, occasional, 18008, 18390.

ONAGRACEAE Dum.—Evening-primrose Family

Circaea alpina L. Enchanter's nightshade.—Wet woods, Tower, August 17, 1889, *E. J. H.*

Epilobium adenocaulon Haussk.—Moist ground, often on lake shores, common, 18343, 18409, 18488, 18525, 18659, 18672, 18776.

Epilobium angustifolium L. Fireweed.—Roadside, near Eaglenest Lake, July 16, 1945, *W. V. B.;* open woods, common, 18292, 18737.

Oenothera biennis L. Common Evening-primrose.—In field, Eaglenest Lake, August 26, 1945, *W. V. B.;* gravelly soil in open woods, or along roads, or gravelly lake shores, 18203, 18512, 18662.

HALORAGIDACEAE R. Br.—Water-milfoil Family

Myriophyllum spicatum L.—In shallow water, Eaglenest Lake, August 30, 1945, *W. V. B.*; lakes, 18076, 18453, 18539.

Myriophyllum alterniflorum DC.—Shallow water, Eaglenest Lake, August 3, 1945, *W. V. B.*; lakes, common, 18537.

Myriophyllum tenellum Bigel.—Shallow margins of lakes, rare, 18523, 18550.

CORNACEAE Link.—Dogwood Family

Cornus canadensis L. Bunchberry.—Coniferous woods, very common, 18060, 18162, 18190, 18529.

Cornus baileyi Coult. & Evans. Bailey's Doogwood.—Bank of stream, Tower, August 17, 1889, *E. J. H.* (reported as *C. sericea*); wet ground in woods, common, 18020, 18078, 18260, 18337.

Cornus rugosa Lam. Round-leaved Dogwood.—Woods, common, 18045, 18414, 18720.

ARALIACEAE Vent.—Ginseng Family

Aralia hispida Vent. Bristly Aralia.—In shallow soil near Eaglenest Lake, July 17, 1945, *W. V. B.*; rocky woods, local, 18122, 18423.

Aralia nudicaulis L. Wild Sarsaparilla.—Eaglenest Lake, July 25, 1945, *W. V. B.*; woods, very common, 18485.

UMBELLIFERAE B. Juss.—Parsley Family

Carum carvi L. Caraway.—Roadside weed, occasional, 18019.

Cicuta maculata L.—Eaglenest Lake, August 2, 1945, *W. V. B.*; wet ground, 18647.

Heracleum lanatum Michx. Cow-parsnip.—Swampy woods, occasional, 18448.

Osmorhiza claytoni (Michx.) Clarke.—Woods, infrequent, 18216.

Pastinaca sativa L. Parsnip.—Roadside weed, not common.

Sanicula marilandica L.—Wet meadows, infrequent, 18306.

Zizia aptera (A. Gray) Fern.—Open woods, rare, 18355.

Zizia aurea (L.) Koch. Golden-alexander.—Rocky woods, frequent, Eaglenest, July 13, 1945, *W. V. B.*; open woods, common, 18286.

ERICACEAE DC.—Heath Family

Andromeda glaucophylla Link. Bog-rosemary.—Sphagnum bogs, 18166.

Arctostaphylos uva-ursi (L.) Spreng. Kinnikinnick.—Forming mats in shallow soil on large flat rocks, Eaglenest Lake, September 6, 1944, *W. V. B.*; rocky woods, common, 18212.

Chamaedaphne calyculata (L.) Moench. Leatherleaf.—Bog, abundant, 18088.

Chimaphila corymbosa Pursh. Pipsissewa.—Coniferous woods, occasional, 18174.

Chiogenes hispidula (L.) T. & G. Moxie-berry.—Coniferous woods, local, 18161.

Gaultheria procumbens L. Creeping Wintergreen.—Woods, Eaglenest Lake, August 29, 1945, *W. V. B.*; coniferous woods, common, 18351, 18433.

Kalmia polifolia Wang.—Sphagnum bogs, 18165.

Ledum groenlandicum Oeder. Labrador-tea.—Bogs and rocky woods, 18274, 18759.

Oxycoccus palustris Pers. Small Cranberry.—Sphagnum bogs, 18097.

Pyrola americana Sweet.—Woods, 18109, 18296, 18356.

Pyrola elliptica Nutt. Shin Leaf.—Woods, 18061, 18159.

Pyrola secunda L.—Woods, 18039, 18134, 18138, 18171.

Pyrola uliginosa Torr. Bog Wintergreen.—Woods, Eaglenest Lake, July 22, 1945, *W. V. B.*; wet ground in woods, 18104.

Pyrola uniflora L. One-flowered Wintergreen.—Woods, 18244.

Pyrola virens Schweigg. (*P. chlorantha* Sw.).—Woods, 18128, 18135; Lake Vermilion, 18611.

Vaccinium angustifolium Ait. Low-bush Blueberry.—Rocky slopes, common, 18030, 18312, 18340.

Vaccinium canadense Kalm. Canada Blueberry.—Rocky woods, 18098.

PRIMULACEAE Vent.—Primrose Family

Lysimachia ciliata L. Fringed Loosestrife.—Wet ground and open woods, not common, 18137.

Lysimachia terrestris (L.) BSP. Swamp-candle.—Swamps and lake shores, common, 18150, 18245.

Lysimachia thyrsiflora L. Tufted Loosestrife.—Shallow water, Eaglenest Lake, July 12, 1945, *W. V. B.*; swamps, rare, 18243.

Trientalis borealis Raf. Star-flower.—Woods, not common, 18170.

OLEACEAE Lindl.—Olive Family

Fraxinus pennsylvanica Marsh. Red Ash.—Low ground, common, 18223, 18404; Lake Vermilion, 18609.

Fraxinus lanceolata Borkh. Green Ash.—Common in alluvial soil; no fruits seen, 18360.

GENTIANACEAE Dum.—Gentian Family

Halenia deflexa (Sm.) Griseb. Spurred Gentian. Woods, occasional, 18053.

APOCYNACEAE Lindl.—Dogbane Family

Apocynum androsaemifolium L. Spreading Dogbane.—Common along roads, Eaglenest Lake, July 8, 1945, *W. V. B.*; open woods, abundant, 18208, 18322, 18531, 18605.

CONVOLVULACEAE Vent.—Morning-glory Family

Convolvulus americanus (Sims) Greene. American Bindweed, 18703.

Convolvulus spithamaeus L. Dwarf Bindweed.—Sandy or rocky soil, occasional, 18235.

POLEMONIACEAE DC.—Phlox Family

Collomia linearis Nutt.—A weed in railroad yards at Ely, 18041.

BORAGINACEAE Lindl.—Borage Family

Cynoglossum boreale Fern. Hound's-Tongue.—Rocky woods, rare; only one plant seen, 18038.

VERBENACEAE J. St. Hil.—Verbena Family

Verbena urticaefolia L. White Vervain.—Dry ground ,infrequent, 18597.

LABIATAE B. Juss.—Mint Family

Agastache anethiodora (Nutt.) Britt.—Open woods, not common, 18334.

Galeopsis tetrahit L.—Burned-off places in woods, occasional, 18451, 18532, 18724.

Lycopus americanus Muhl. Water Horehound.—Gravelly shores, abundant, 18131, 18367, 18701, 18727.

Lycopus uniflorus Michx.—Eaglenest Lake, July 28, 1945, *W. V. B.*; wet ground in woods, 18579, 18679, 18748; wet ground, Tower, 18620.

Mentha canadensis L. Common Mint. Gravelly shores, common, 18084, 18129, 18200, 18408, 18587; shore of Lake Vermilion, 18606 .

Monarda menthaefolia Benth.—In waste field, Eaglenest Lake, September 7, 1945, *W. V. B.*; grassy hillside, not common, 18769, 18723.

Prunella vulgaris L. Selfheal.—Open woods, common, 18012.

Scutellaria epilobiifolia A. Hamilt. Skullcap.—Gravelly shores, common, 18127, 18521.

Scutellaria lateriflora L.—On rocky island in Eaglenest Lake, August 29, 1945, *W. V. B.*; gravelly shores, common; often growing with *S. epilobiifolia*, 18082, 18132, 18520.

Stachys borealis Rydb.—Edge of meadow, rare, 18262.

Stachys brevidens Rydb.—Tower, *O. Lugger* in 1891, *fide* Rydberg in Brittonia 1: 95 (1931).

SOLANACEAE Pers.—Nightshade Family

Physalis virginiana Mill.—Virginia Ground-cherry.—Sandy soil, occasional, 18284, 18468.

SCROPHULARIACEAE Lindl.—Figwort Family

Chelone glabra L.—Turtlehead.—Meadow, near Tower, 18618.

Linaria vulgaris Mill. Butter-and-Eggs.—Observed in Tower.

Melampyrum lineare Desr. Cow-wheat.—Rocky ridge, Eaglenest Lake, July 12, 1945, *W. V. B.*; moist ground in woods, rare, 18169, 18420.

Mimulus ringens L.—In shallow running water, Eaglenest Lake, July 22, 1945, *W. V. B.*; swampy ground in woods, 18242.

Scrophularia lanceolata Pursh. (*S. leporella* Bickn.).—Open woods, and thickets, very common; plants 1.5-2 m. tall, 18271, 18457.

Verbascum thapsus L. Common Mullein.—Gravelly roadsides and banks, not common, 18673.

Veronica serpyllifolia L. Thyme-leaved Speedwell.—Wet ground along trail in woods, Ely, 18627.

Veronicastrum virginicum (L.) Farw. Culver-root.—In burned-over places near Eaglenest Lake, August 9, 1945, *W. V. B.*; moist woods, occasional, 18293.

PLANTAGINACEAE Lindl.—Plantain Family

Plantago major L. Broad-leaved Plantain.—Common weed in waste ground and along paths, 18374, 18463.

RUBIACEAE B. Juss.—Madder Family

Galium asprellum Michx. Rough Bedstraw.—Alluvial soil in thickets, not common, 18275, 18276.

Galium boreale L. Northern Bedstraw.—Rocky soil in open woods, infrequent, 18456.

Galium tinctorium L. (*G. claytoni* Michx.).—Gravelly shores, common, 18133, 18275, 18461, 18527.

Galium triflorum Michx. Sweet-scented Bedstraw.—Woods, common, 18218, 18220.

Galium vaillantii DC.—Woods, 18168.

CAPRIFOLIACEAE Vent.—Honeysuckle Family

Diervilla lonicera Mill. Bush Honeysuckle.—Most plentiful shrub at Eaglenest Lake, July 8, 1945, *W. V. B.*; rocky woods, abundant, 18029.

Linnaea americana Forbes. Twinflower.—Woods, Eaglenest Lake, July 2, 1945, *W. V. B.*; rocky woods, common, 18052.

Lonicera canadensis Marsh. American Fly Honeysuckle.—Open woods, common, 18726.

Lonicera glaucescens Rydb.—Open rocky woods, infrequent. Eaglenest Lake, July 13, 1945, *W. V. B.*

Lonicera hirsuta Eat.—Rocky woods, infrequent, Eaglenest Lake, July 12, 1945, *W. V. B.*; open woods, occasional, 18125, 18259.

Sambucus pubens Michx. Red Elder.—Wet ground, common, 18048, 18412, 18467, 18555.

Symphoricarpos occidentalis Hook. Wolfberry.—Rocky open woods, infrequent, 18483.

Viburnum affine Bush.—Rocky woods, occasional, 18247, 18504.

Viburnum rafinesquianum Schult.—Rocky woods, not uncommon, 18042.

Viburnum trilobum Marsh.. American Cranberry-bush.—Eaglenest Lake, September 5, 1945, *W. V. B.*

CAMPANULACEAE Juss.—Bellflower Family

Campanula uliginosa Rydb.—Wet ground, Eaglenest Lake, July 28, 1945, *W. V. B.*; swamps and shores, 18085, 18194, 18383, 18425, 18477.

LOBELIACEAE Dum.—Lobelia Family

Lobelia dortmanna L. Water Lobelia—Leaves flat, not terete as they are said to be in Gray's *New Manual*. In shallow water in lakes, not uncommon, 18428, 18524.

COMPOSITAE Adans.—Composite Family

Achillea millefolium L. Yarrow.—Open places, Eaglenest Lake, August 2, 1945, *W. V. B.*; roadsides, meadows, open woods, very common, 18373.

Ambrosia elatior L. Common Ragweed.—Open space, Eaglenest Lake, August 26, 1945, *W. V. B.*; sandy roadsides, not yet common, 18379, 18468.

Ambrosia trifida L. Giant Ragweed.—Waste places, occasional, Ely, 18496.

Anaphalis margaritacea (L.) A. Gray. Pearly Everlasting—Open woods, common, 18509, 18714.

Antennaria neglecta Greene.—Rocky hillsides, frequent, 18641.

Antennaria neodioica Greene.—On sunny rocky slopes, Eaglenest Lake, August 29, 1945, *W. V. B.*

Antennaria plantaginifolia (L.) Hook.—Rocky open woods, common, 18254.

Anthemis cotula L. Dog-fennel.—Waste places, occasional, Ely, 18497.

Arctium minus (Hill) Bernh. Burdock.—An occasional weed in waste ground, 18773.

Artemisia absinthium L. Common Wormwood or Absinth.—Gravelly soil along roads; about 1 m. tall, in clumps, Ely, 18491, 18633.

Artemisia gnaphalodes Nutt.—Eaglenest in 1945, *W. V. B.*; gravelly hillsides, local, 18709; gravelly "prairie," Ely, 18630.

Aster macrophyllus L. Large-leaved Aster.—Along wooded road, Eaglenest Lake, August 25, 1945, *W. V. B.*; woods, very common, 18691, 18740.

Aster paniculatus Lam.—Roadside, Ely, September 7, 1945, *W. V. B.*; low grassy places, Eaglenest Lake, July 28, 1945, *W. V. B.*; meadows, common, 18684, 18765.

Aster puniceus L.—Swampy ground, occasional, 18585, 18697.

Aster sagittifolius Willd.—Eaglenest Lake, August 2, 1945, *W. V. B.*

Aster umbellatus Mill. Flat-top Aster.—Roadside, Eaglenest Lake, August 13, 1945, *W. V. B.*; meadows and open woods, common, 18600, 18713, 18736.

Bidens cernua L. Nodding Bur-marigold.—Moist places, Eaglenest Lake, August 24, 1945, *W. V. B.*; wet ground, common, 18686, 18753.

Bidens frondosa L. Beggar-ticks.—Gravelly shore of lake, 18672, 18767.

Chrysanthemum leucanthemum L. Ox-eye Daisy..—Waste places, Eaglenest Lake, July 13, 1945, *W. V. B.*; gravelly soil, common.

Chrysopsis villosa (Pursh) Nutt. Golden-aster.—Dry gravelly soil, Ely, 18729.

Cirsium arvense (L.) Scop. Canada Thistle.—Common weed of roadsides and waste places, 18487, 18554.

Cirsium muticum Michx. Swamp Thistle.—Waste places, Eaglenest Lake, August 13, 1945, *W. V. B.;* woods and meadows, occasional, 18136, 18473; Tower, 18621.

Cirsium vulgare (Savi) Airy-Shaw. Bull Thistle.—An occasional weed in waste ground, 18758.

Erigeron canadensis L. Horseweed.—Waste places, occasional.

Erigeron philadelphicus L. —Moist ground, occasional, 18094.

Erigeron strigosus Muhl. Daisy Fleabane.—Eaglenest Lake, August 26, 1945, *W. V. B.;* dry open woods ,and roadsides, common, 18430.

Eupatorium maculatum L. Roadsides, Eaglenest Lake, August 13, 1945, *W. V. B.;* wet ground, common, 18698, 18708, 18734.

Eupatorium perfoliatum L. Boneset.—Meadows and edges of woods, common and conspicuous, 18439, 18766.

Gnaphalium macounii Greene.—Open woods, not common, 18674.

Gnaphalium uliginosum L.—Eaglenest Lake, in 1945, *W. V. B.;* moist ground, occasional, 18315.

Grindelia squarrosa (Pursh) Dunal.—Gravelly soil, Ely, 18631.

Helianthus maximiliani Schrad.—Gravelly railroad embankment, local, 18594, 18717.

Heliopsis helianthoides (L.) Sweet. Sunflower Heliopsis.—Eaglenest Lake, July 11, 1945, and July 24, 1945, *W. V. B.;* rocky hillsides, and gravelly soil along roads, occasional, 18016.

Hieracium canadense Michx. Canada Hawkweed.—Eaglenest Lake, August 2, 1945; also September 7, 1945, *W. V. B.;* dry woods and thickets, common, 18139, 18580, 18738.

Hieracium scabrum Michx.—Dry woods, common, 18722, 18735, 18760, 18763.

Iva xanthifolia Nutt. Horseweed.—Weed in waste ground, Ely, 18568.

Lactuca canadensis L. Wild Lettuce.—Roadsides, hillsides, open woods, common, 18153, 18395, 18581, 18671.

Lactuca floridana (L.) Gaertn.—Open woods, common, 18450, 18514, 18749.

Lactuca pulchella (Pursh.) DC.—Rocky hillsides, local, 18105, 18391, 18751.

Lactuca scariola L. Prickly Lettuce.—Weed along street in Ely, 18565.

Matricaria matricarioides (Less.) Porter. Pineapple-weed.—Dooryard weed, common, 18405.

Petasites palmatus (Ait.) A. Gray. Sweet Coltsfoot.—Wet ground in woods, common, 18354.

Ratibida pinnata (Vent.) Barnh.—Field, Eaglenest Lake, August 26, 1945, *W. V. B.;* gravelly soil in open woods, local, 18295, 18718.

Rudbeckia hirta L. Black-eyed Susan.—Along railroad, Eaglenest Lake, July 11, 1945; also July 24, 1945, *W. V. B.;* gravelly banks, and along roads, 18372, 18424.

Senecio congestus (R. Br.) DC.—Gravelly shores, not common, 18024, 18657.

Senecio pauperculus Michx.—Open woods, Eaglenest Lake, July 10, 1945, *W. V. B.;* grassy banks and open woods, common, 18034, 18273.

Solidago canadensis L.—Meadows and open woods, common, 18475, 18507, 18598, 18516, 18656, 18678.

Solidago graminifolia (L.) Salisb.—Sandy lake shores and edges of woods, common, 18368, 18478, 18651, 18719.

Solidago hispida Muhl.—Dry open woods, common, 18251, 18655, 18755.

Solidago juncea Ait. Early Goldenrod.—Eaglenest Lake, August 26, 1945, *W. V.*

B.; open woods, and roadsides, common, beginning to flower about the middle of July, 18329, 18362, 18593, 18699.

Solidago nemoralis Ait.—Open field, Eaglenest Lake, August 26, 1945, *W. V. B.*; sandy soil in woods, common, 18251.

Solidago serotina Ait. Late Goldenrod.—Woods, common, 18603.

Solidago speciosa Nutt.—Open woods, 18506.

Solidago uliginosa Nutt. Swamp Goldenrod.—Swamps and meadows, 18653, 18742.

Sonchus asper (L.) Hill. Spiny Sow-thistle.—Waste ground, occasional, Ely, 18569.

Sonchus uliginosus Bieb.—Roadsides and waste places, not uncommon; 1-2 m. tall, 18479, 18650.

Tanacetum vulgare L. Tansy.—Occasional weed in waste places in towns; Ely, 18571.

Taraxacum officinale Weber. Common Dandelion.—Common weed in waste ground and along roads, 18387, 18707.

Tragopogon dubius Scop. Yellow Oyster-plant.—Sandy soil on railroad embankment, not common, 18079, 18319.

Tragopogon pratensis L. Goat's-beard.—Weed in waste places, Ely, 18573.

After the data in this paper were assembled in 1946, Dr. R. M. Tryon kindly sent me a copy of his and Dr. J. W. Moore's check list of Minnesota plants.* A comparison of my list with theirs, after making due allowance for slight nomenclatural differences, shows that seventeen plants are listed as occurring in St. Louis County whose names are either not attributed to Minnesota or are not accounted for in synonymy. This suggests the possibility that all or some of the following plants may be regarded as new or additional records for Minnesota: *Woodsia oregana, Agropyron subsecundum, Carex grayii, Eleocharis palustris, Eriophorum tenellum, Streptopus roseus, Salix vitellina, Rumex occidentalis, Chenopodium hybridum, Rorippa hispida, Geum strictum, Viola canadensis, Viola palustris, Viola subvestita, Stachys borealis, Galium vaillantii.*

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* Moore, J. W., & Tryon, R. M., A preliminary check list of the flowering plants, ferns, and fern-allies of Minnesota. 99 pp. (mimeographed). Nicholson Hall Bookstore, University of Minnesota, Minneapolis. 1946. Price $1.00.

Studies in the Comparative Anatomy of the Ericales
II. Ericaceae—Subfamily Arbutoideae

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In the preceding paper of this series (Cox, 1948) representative species of nineteen genera of the Rhododendroideae were evaluated on the basis of anatomical and morphological characteristics. Considerable light was thus shed on the phylogenetic relationships between these genera. This paper is a continuation of the series in which genera comprising the subfamily Arbutoideae are also evaluated according to the same criteria used in the study of the Rhododendroideae.

Subfamily ARBUTOIDEAE

Flowers normally pentamerous, sometimes tetramerous. Calyx of 4-5 separate or fused sepals, entirely free from the ovary or partially fused with it. Corolla of 4-5 fused petals, mostly bell-, flask- or pitcher-shaped and generally deciduous. Stamens 8-10, with or without awns. Ovary superior, of 2-10 cells. Fruit a 2-5 celled loculicidal capsule, a capsule enclosed by and fused with the fleshy calyx, a 4-10 seeded berry-like drupe, or a few to many seeded berry. Wood diffuse porous to semi-ring porous. Vessel walls thin or thick, even or uneven. Vessels angular in outline. Vessel end wall angle less than 30°. Vessel perforation plates scalariform with many bars to porous. Vessel side wall pits half-bordered, scalariform to round opposite. Tertiary thickening of the vessel walls absent, or present as a delicate to heavy spiral reticulum. Medullary rays Heterogeneous Types I, IIA, or IIB.

Drude (1889) recognizes 17 genera in this subfamily grouped in three tribes defined primarily on the character of the fruit. In the present study *Chamaedaphne*, which Drude incorporates in *Lyonia*, is considered a distinct genus, while the remaining species of *Lyonia* studied are placed in the genus *Andromeda*. No material of *Orphanidesia* has been available. Therefore this study is based on representative species of sixteen genera of the Arbutoideae. They are: *Enkyanthus* Lour., *Cassiope* Don, *Leucothoe* Don, *Andromeda* L., *Agauria* DC., *Oxydendron* DC., *Epigaea* L., *Chamaedaphne* Mnch., *Gaultheria* L., *Diplycosia* Bl., *Pernettya* Gaud., *Chiogenes* Salisb., *Wittsteinia* F.v.M., *Arbutus* L., *Arctostaphylos* Adans., and *Arctous* Gray.

Based on floral structure, fruit structure and wood anatomical characteristics these sixteen genera appear to fall naturally into five more or less well defined groups. These are recognized as tribes. Two of these tribes, the Gaultherieae and Arbuteae, have the same generic complements established by Drude but his Andromedeae is here divided into three smaller tribes.

Tribe VI Cassiopeae Tribus nova

Figs. 1, 6

Flowers typically pentamerous. Corolla deciduous. Calyx entirely free from the ovary. Anthers opening by terminal pores or clefts. Fruit a dry, 5-celled, flattened-globose, loculicidal capsule. Wood diffuse porous. Vessels angular in outline with thin

Fig. 1

TRIBE VI CASSIOPEAE

	WOOD TYPE: DIFFUSE POROUS	WOOD TYPE: SEMI-RING POROUS	VESSEL OUTLINE	VESSEL WALL: THIN	VESSEL WALL: THICK	VESSEL WALL: EVEN	VESSEL WALL: UNEVEN	VESSEL DIMENSIONS: DIAMETER	VESSEL DIMENSIONS: LENGTH	VESSEL DIMENSIONS: RATIO	VESSEL PERFORATION: % SCALAR. 9+ BARS	VESSEL PERFORATION: % SCALAR. 8- BARS	VESSEL PERFORATION: % POROUS	VESSEL PERFORATION: AVERAGE NO. BARS SCAL.	VESSEL SIDE WALL PITS: SCALARIFORM	VESSEL SIDE WALL PITS: ELONGATE-ELLIPTIC	VESSEL SIDE WALL PITS: ROUND	WOOD PARENCHYMA PITS: SCALARIFORM	WOOD PARENCHYMA PITS: ELONGATE-ELLIPTIC	WOOD PARENCHYMA PITS: ROUND	3RD THICK.: ABSENT	3RD THICK.: PRESENT	MED. RAY TYPE: HET. I	MED. RAY TYPE: HET. IIA	MED. RAY TYPE: HET. IIB
ENKYANTHUS	■		ANGULAR	■			■	23.9	678.3	1:28	100	0	0	34.7		■	■		■	■		■			■	
CASSIOPE	■			■			■	17.0	216.2	1:13	42	53	5	7.1		■	■		■	■		■			■	
EPIGAEA	■			■			■	23.2	320.9	1:14	18	68	14	7.2		■	■		■	■		■			■	
AGAURIA	■			■			■	26.6	277.9	1:10	11	39	50	5.2		■	■		■	■		■			■	

Fig. 2

TRIBE VII GAULTHERIEAE

	WOOD TYPE: DIFFUSE POROUS	WOOD TYPE: SEMI-RING POROUS	VESSEL OUTLINE	VESSEL WALL: THIN	VESSEL WALL: THICK	VESSEL WALL: EVEN	VESSEL WALL: UNEVEN	VESSEL DIMENSIONS: DIAMETER	VESSEL DIMENSIONS: LENGTH	VESSEL DIMENSIONS: RATIO	VESSEL PERFORATION: % SCALAR. 9+ BARS	VESSEL PERFORATION: % SCALAR. 8- BARS	VESSEL PERFORATION: % POROUS	VESSEL PERFORATION: AVERAGE NO. BARS SCAL.	VESSEL SIDE WALL PITS: SCALARIFORM	VESSEL SIDE WALL PITS: ELONGATE-ELLIPTIC	VESSEL SIDE WALL PITS: ROUND	WOOD PARENCHYMA PITS: SCALARIFORM	WOOD PARENCHYMA PITS: ELONGATE-ELLIPTIC	WOOD PARENCHYMA PITS: ROUND	3RD THICK.: ABSENT	3RD THICK.: PRESENT	MED. RAY TYPE: HET. I	MED. RAY TYPE: HET. IIA	MED. RAY TYPE: HET. IIB
DIPLYCOSIA	■		ANGULAR		■		■	24.4	628.8	1:26	100	0	0	35.0		■			■			■			■	
WITTSTEINIA	■				■		■	20.3	464.3	1:23	100	0	0	33.7		■			■			■			■	
CHIOGENES				■		■		22.8	262.5	1:12	16	84	0	6.2		■			■			■			■	
GAULTHERIA	■				■		■	25.2	401.1	1:16	80	20	0	12.4		■	■		■	■		■			■	
PERNETTYA	■				■		■	24.0	376.6	1:16	81	18	1	12.9		■	■		■	■		■			■	

Fig. 3

TRIBE VIII ANDROMEDEAE

	WOOD TYPE: DIFFUSE POROUS	WOOD TYPE: SEMI-RING POROUS	VESSEL OUTLINE	VESSEL WALL: THIN	VESSEL WALL: THICK	VESSEL WALL: EVEN	VESSEL WALL: UNEVEN	VESSEL DIMENSIONS: DIAMETER	VESSEL DIMENSIONS: LENGTH	VESSEL DIMENSIONS: RATIO	VESSEL PERFORATION: % SCALAR. 9+ BARS	VESSEL PERFORATION: % SCALAR. 8- BARS	VESSEL PERFORATION: % POROUS	VESSEL PERFORATION: AVERAGE NO. BARS SCAL.	VESSEL SIDE WALL PITS: SCALARIFORM	VESSEL SIDE WALL PITS: ELONGATE-ELLIPTIC	VESSEL SIDE WALL PITS: ROUND	WOOD PARENCHYMA PITS: SCALARIFORM	WOOD PARENCHYMA PITS: ELONGATE-ELLIPTIC	WOOD PARENCHYMA PITS: ROUND	3RD THICK.: ABSENT	3RD THICK.: PRESENT	MED. RAY TYPE: HET. I	MED. RAY TYPE: HET. IIA	MED. RAY TYPE: HET. IIB
ANDROMEDA	■		ARCEOUS	■			■	27.4	414.9	1:15	100	0	0	20.7		■	■		■	■		■			■	
LEUCOTHOE	■			■			■	25.9	390.8	1:15	93	7	0	16.1		■	■		■	■		■			■	
CHAMAEDAPHNE	■			■			■	20.2	282.4	1:14	80	20	0	10.7		■	■		■	■		■			■	

Fig. 4

TRIBE IX OXYDENDREAE

	WOOD TYPE: DIFFUSE POROUS	WOOD TYPE: SEMI-RING POROUS	VESSEL OUTLINE	VESSEL WALL: THIN	VESSEL WALL: THICK	VESSEL WALL: EVEN	VESSEL WALL: UNEVEN	VESSEL DIMENSIONS: DIAMETER	VESSEL DIMENSIONS: LENGTH	VESSEL DIMENSIONS: RATIO	VESSEL PERFORATION: % SCALAR. 9+ BARS	VESSEL PERFORATION: % SCALAR. 8- BARS	VESSEL PERFORATION: % POROUS	VESSEL PERFORATION: AVERAGE NO. BARS SCAL.	VESSEL SIDE WALL PITS: SCALARIFORM	VESSEL SIDE WALL PITS: ELONGATE-ELLIPTIC	VESSEL SIDE WALL PITS: ROUND	WOOD PARENCHYMA PITS: SCALARIFORM	WOOD PARENCHYMA PITS: ELONGATE-ELLIPTIC	WOOD PARENCHYMA PITS: ROUND	3RD THICK.: ABSENT	3RD THICK.: PRESENT	MED. RAY TYPE: HET. I	MED. RAY TYPE: HET. IIA	MED. RAY TYPE: HET. IIB
OXYDENDRON	■		ANGULAR	■			■	31.1	433.2	1:12	7	23	70	5.1		■	■		■	■		■			■	

Fig. 5

TRIBE X ARBUTEAE

	WOOD TYPE: DIFFUSE POROUS	WOOD TYPE: SEMI-RING POROUS	VESSEL OUTLINE	VESSEL WALL: THIN	VESSEL WALL: THICK	VESSEL WALL: EVEN	VESSEL WALL: UNEVEN	VESSEL DIMENSIONS: DIAMETER	VESSEL DIMENSIONS: LENGTH	VESSEL DIMENSIONS: RATIO	VESSEL PERFORATION: % SCALAR. 9+ BARS	VESSEL PERFORATION: % SCALAR. 8- BARS	VESSEL PERFORATION: % POROUS	VESSEL PERFORATION: AVERAGE NO. BARS SCAL.	VESSEL SIDE WALL PITS: SCALARIFORM	VESSEL SIDE WALL PITS: ELONGATE-ELLIPTIC	VESSEL SIDE WALL PITS: ROUND	WOOD PARENCHYMA PITS: SCALARIFORM	WOOD PARENCHYMA PITS: ELONGATE-ELLIPTIC	WOOD PARENCHYMA PITS: ROUND	3RD THICK.: ABSENT	3RD THICK.: PRESENT	MED. RAY TYPE: HET. I	MED. RAY TYPE: HET. IIA	MED. RAY TYPE: HET. IIB
ARCTOUS		■	ARCEOUS		■		■	26.0	193.2	1:8	0	100	0	3.0		■	■		■	■		■			■	
ARBUTUS	■	■			■		■	26.0	250.6	1:10	0	36	64	2.5		■	■		■	■		■			■	
ARCTOSTAPHYLOS	■				■		■	26.4	304.5	1:11	0	6	94	2.1		■	■		■	■		■			■	

Figs. 1-5. Summary of significant wood anatomical data for genera of the five tribes of the Arbutoideae.

and even walls. Vessel perforation plates scalariform with many bars or transitional from that state to porous, none strictly porous. Vessel side wall pits scalariform, elongate-elliptic opposite to round opposite. Tertiary thickening of vessel walls absent. Medullary rays Heterogeneous Type I.

This tribe is composed of four genera: *Enkyanthus, Cassiope, Epigaea* and *Agauria*. Its members form a natural, closely related group easily distinguishable from the subfamily's other tribes by the combination of two characteristics: absence of vessel wall tertiary thickening and dry capsular fruit.

Enkyanthus is unquestionably the most primitive member of the tribe as well as of the entire subfamily. The extremely long vessels, the high vessel diameter to length ratio, the absence of porous vessel perforations and the large average number of bars to the scalariform perforations are specific examples of primitiveness in this genus (figure 1). Other anatomical characteristics such as vessel wall outline, medullary ray type, etc., as well as details of floral and fruit structure establish further its primitiveness. In addition, anatomical variation within the genus is great, as may be seen from the following table, and the more primitive species of *Enkyanthus* are without doubt the most primitive in the Ericaceae.

ENKYANTHUS Lour.

Tabulation of anatomical characteristics varying between the species.

E. quinqueflorus Lour., (NY—Tsang 24344); *E. deflexus* (Griff.) Schneid. (NY—Rock 18426); *E. nikkoensis* Maxim. (NY—Faurie 5823).

	E. quinqueflorus	*E. deflexus*	*E. nikkoensis*
Vessel diameter			
Maximum	42μ	47μ	29μ
Minimum	13μ	15μ	8μ
Average	25.7μ	28.2μ	17.9μ
Vessel length			
Maximum	998μ	1154μ	829μ
Minimum	503μ	283μ	354μ
Average	753.8μ	709.2μ	571.9μ
Ratio	1:29	1:25	1:32
Perforation plate			
% scalariform 9+ bars	100	100	100
% scalariform 8— bars	0	0	0
% porous	0	0	0
Average no. of bars	41.4	32.7	30.0

Considerably more advanced than *Enkyanthus* but obviously related to it is *Cassiope*. This genus of some seven high northern, boreal species may occasionally have tetramerous flowers. However its many-seeded, dry, loculicidal capsule quite closely resembles the capsule of *Enkyanthus*, even though the latter normally produces a one-seeded capsule through abortion of all but

one of the numerous ovules. Significant also are the many close similarities in secondary wood structure. Wood of both genera is diffuse porous. Vessel walls in both are thin and even and vessels are angular in outline. Heterogeneous Type I medullary rays are common to both. In addition their vessel side wall and wood parenchyma pitting is similar (figure 1).

Cassiope's advance over *Enkyanthus* appears not only in its occasional tetramerous floral structure but in anatomical details as well. The most apparent of these are the shortening in average vessel length with consequent reduction in vessel diameter to length ratio, and the great decrease in average number of bars in the vessel perforation plates. Although as a genus it is essentially non-porous, one species has managed to evolve a relatively small percentage of porous vessel perforations. The four species studied show less anatomical variation than *Enkyanthus*, as may be determined by a comparison of the following table with similar data already presented for *Enkyanthus*.

CASSIOPE Don

Tabulation of anatomical characteristics varying between the species.

C. stellariana Coville (NY—Walker 969); *C. lycopodioides* (Pall.) Don (NY—Eyerdam 2138); *C. mertensiana* (Bong.) Don (NY—Hulten 8238); *C. tetragona* (L.) Don (NY—herbarium sheet identified only by "Peary voyage to Arctic in 1896").

	C. stellariana	*C. lycopodioides*	*C. mertensiana*	*C. tetragona*
Vessel diameter				
Maximum	15μ	34μ	36μ	28μ
Minimum	5μ	10μ	10μ	10μ
Average	10.1μ	18.4μ	22.5μ	17.0μ
Vessel length				
Maximum	262μ	333μ	467μ	276μ
Minimum	85μ	127μ	184μ	99μ
Average	177.7μ	219.4μ	298.7μ	169.2μ
Ratio	1:18	1:12	1:13	1:10
Perforation plate				
% scalariform 9+ bars	80	90	0	0
% scalariform 8— bars	20	10	80	100
% porous	0	0	20	0
Average no. of bars	10.8	10.1	2.8	4.6

Epigaea is an interesting genus of discontinuous distribution. One of its two species, *E. repens*, is typically eastern North American while the other, *E. asiatica*, is typically east Asiatic. Morphologically the two species are quite similar. They are small, prostrate and broad-leaved. Their pentamerous flowers are often heteromorphous. The 5-lobed, 5-celled, many-ovuled ovary develops into a depressed-globose, 5-celled, loculicidal capsule with many seed. The relationship of *Epigaea* to *Enkyanthus* and *Cassiope* seems unquestionable.

From an anatomical standpoint this close relationship is also apparent (figure 1). All are similar in such details as type wood, vessel outline and

wall structure, medullary ray type and vessel side wall pitting. It is interesting to note that one of *Epigaea's* two species has developed porous vessel perforations to a limited extent while the other has not. This is similar to the condition in *Cassiope* and the two genera are considered to be at about the same level of advancement.

EPIGAEA L.

Tabulation of anatomical characteristics varying between the species.

E. repens L. (NC—herbarium sheet 6252); *E. asiatica* Maxim. (NY—Faurie 615).

	E. asiatica	*E. repens*
Vessel diameter		
Maximum	31μ	42μ
Minimum	13μ	10μ
Average	22.5μ	23.9μ
Vessel length		
Maximum	446μ	460μ
Minimum	184μ	148μ
Average	307.8μ	334.0μ
Ratio	1:14	1:14
Vessel perforation		
% scalariform 9+ bars	27	10
% scalariform 8— bars	73	63
% porous	0	27
Average no. of bars	6.9	5.5

The most advanced genus of the Cassiopeae is *Agauria* whose five species inhabit Madagascar, Mauritius and mountainous regions of tropical Africa. Morphologically and anatomically *Agauria* differs in no important respects from its kindred genera (figure 1). Its relative advancement is attested by the relatively low vessel diameter to length ratio and by the presence of porous perforations in vessels of all species studied. Also the proportion of many-barred scalariform perforation plates is quite low. Anatomical variation within the genus is shown below:

AGAURIA DC.

Tabulation of anatomical characteristics varying between the species.

A. salicifolia (Lam.) Hook. f. (NY—no data); *A. polyphylla* Baker (US—Schufeldt no number, Herbarium sheet 156136); *A. angustifolia* (NY—no data).

	A. salicifolia	*A. polyphylla*	*A. angustifolia*
Vessel diameter			
Maximum	33μ	42μ	52μ
Minimum	11μ	20μ	13μ
Average	22.3μ	27.0μ	30.6μ
Vessel length			
Maximum	418μ	467μ	396μ

AGAURIA DC. (*Continued*)

	A. salicifolia	*A. polyphylla*	*A. angustifolia*
Minimum	135μ	142μ	149μ
Average	307.8μ	274.6μ	251.2μ
Ratio	1:14	1:10	1:8
Perforation plate			
% scalariform 9+ bars	14	6	14
% scalariform 8— bars	26	47	43
% porous	60	47	43
Average no. of bars	5.8	3.5	6.2

The Cassiopeae as a tribe is relatively primitive. The dry capsular fruit, the absence of even partial fusion of calyx with ovary, the absence of vessel wall tertiary thickening, the retention of scalariform vessel side wall pits and the presence of Heterogeneous Type I medullary rays all represent primitive characteristics in this subfamily.

Phylogenetic development within the tribe probably stemmed from one of the lower species of *Enkyanthus* (figure 6). This developmental line was characterized primarily by reduction of vessel diameter to length ratio, reduction in number of bars comprising the vessel perforation plates and gradual acquisition of porous perforation plates. Arising succeessively from this phyloegnetic line are *Cassiope*, *Epigaea* and *Agauria*, with some species of the last named genus representing today the highest development reached by the Cassiopeae.

Tribe VII GAULTHERIEAE Niedenz.

Figs. 2, 6

Flowers pentamerous or tetramerous. Calyx free from the ovary, or partially to almost completely fused with it. Anthers opening by terminal pores. Ovary 2-5 celled. Fruit a loculicidal capsule partially to almost completely enveloped by the persistent fleshy calyx, or a juicy berry. Wood diffuse porous. Vessels angular in outline with thin to moderately thick, even to slightly uneven walls. Vessel perforation plates scalariform to rarely porous. Vessel side wall pits scalariform to round opposite. Tertiary thickening of the vessel walls absent or present. Medullary rays Heterogeneous Type I.

The five genera constituting this tribe (*Diplycosia, Wittsteinia, Chiogenes, Gaultheria* and *Pernettya*) all are characterized by their tendency to form fleshy, berry-like fruits and the virtual absence of porous perforations. On strictly anatomical grounds this tribe could perhaps be divided into two smaller groups (figure 2) with *Diplycosia* and *Wittsteinia* being placed in one, *Gaultheria* and *Pernettya* in the second, and *Chiogenes* occupying a rather confusing intermediate position. However the basic similarity in fruit structure in these genera is thought to be of sufficient importance to retain all within a single tribe as does Drude.

Diplycosia and *Wittsteinia* are so similar in anatomical structure that they may be considered together. As is shown in figure 2, the only significant anatomical difference between the two genera is in vessel dimensions with *Wittsteinia* occupying a more advanced position in this respect.

This view that *Wittsteinia* is more advanced than *Diplycosia* is corroborated by morphological evidence. *Wittsteinia's* 2-3 celled ovary is of course more advanced than *Diplycosia's* 5-celled ovary. Also the mature fruit of *Wittsteinia* is more "berry-like", i.e. exhibits more complete fusion of fleshy calyx and capsule, than is the fruit of *Diplycosia*. Thus it is clear that monotypic *Wittsteinia* (*Wittsteinia vacciniacea* F.v.M.; CAL—herbarium sheet 22262) is a derived genus and undoubtedly descended from *Diplycosia*.

DIPLYCOSIA Bl.

Tabulation of anatomical characteristics varying between the species.

D. cinnamomifolia Stapf. (NY—Clemens 29112); *D. luzonica* (A. Gray) Merr. (NY—Williams 1341; US—herbarium sheet 40397); *D. ciliolata* Hook. f. (NY—Clemens 30084).

	D. cinnamomifolia	*D. luzonica*	*D. ciliolata*
Vessel diameter			
Maximum	31μ	42μ	29μ
Minimum	13μ	15μ	13μ
Average	24.6μ	26.8μ	21.9μ
Vessel length			
Maximum	1125μ	779μ	779μ
Minimum	446μ	425μ	170μ
Average	819.7μ	610.8μ	455.8μ
Ratio	1:33	1:23	1:21
Perforation plate			
% scalariform 9+ bars	100	100	100
% scalariform 8— bars	0	0	0
% porous	0	0	0
Average no. of bars	42.0	34.8	28.2

Chiogenes, *Gaultheria* and *Pernettya* form a group of genera related not only by floral and fruit structure but by certain anatomical characteristics as well. The fruit of *Chiogenes* and *Gaultheria* is a "berry" formed by the fusion of the fleshy calyx with the capsule. In *Pernettya* the calyx remains free of the ovary which develops into a juicy, fleshy berry.

Anatomically all three genera are similar in details of wood type, vessel outline and wall structure, vessel perforation structure, vessel side wall and wood parenchyma pitting, and medullary ray type (figure 2). *Chiogenes* (*C. hispidula* Torr. & Gray; NC—herbarium sheet 8961), however, lacks tertiary vessel wall thickening, a characteristic it shares with *Diplycosia* and *Wittsteinia*, while *Gaultheria* and *Pernettya* have developed a moderately heavy spiral-reticulum of tertiary thickening. *Pernettya* is the only member of the tribe to have developed porous perforation plates, but only to a very limited extent.

The Gaultherieae is, therefore, composed of two generic groups which undoubtedly arose from a common ancestor. This developmental line is manifested in the gradual formation of a berry-like fruit and finally a berry. Successive branches from the main phylogenetic line set off *Diplycosia, Chiogenes* and *Gaultheria* (figure 6). *Wittsteinia* appears to have been derived directly from *Diplycosia* and *Pernettya* must have arisen from one of the more primitive species of *Gaultheria.*

The *Diplycosia-Wittsteinia* developmental line is characterized by the early acquisition of thicker and more uneven vessel walls than is typical of the tribe's remaining genera. Likewise the loss of scalariform vessel side wall and wood parenchyma pits is the result of an accelerated rate of development in these characteristics. However the absence of porous or even few-barred scalariform perforations, the absence of tertiary thickening of the vessel walls, and the high vessel diameter to length ratio in this generic group establishes its relative primitiveness. The many similarities between *Diplycosia* and *Wittsteinia,* morphologically and anatomically, leads only to the conclusion that, since *Wittsteinia* as a genus is more advanced than *Diplycosia,* the latter must have given rise to *Wittsteinia.*

Chiogenes apparently branched in turn from the phylogenetic line leading to *Gaultheria.* Its development in all characteristics was more orderly. While retaining vessels with thin, even walls, without tertiary thickening, and with some scalariform side wall pits, it has reduced greatly the vessel diameter to length ratio and the average number of bars in its scalariform perforation plates. Considered along with its relatively more advanced fruit these characteristics mark *Chiogenes* as a more advanced genus than either *Diplycosia* or *Wittsteinia.*

The development of *Gaultheria* and *Pernettya* involved further advances in fruit structure and formation of tertiary vessel wall thickening. Again the many basic similarities in morphology and anatomy strongly indicate that *Pernettya* originated from *Gaultheria.* Since the ranges of anatomical variation in these two genera overlap considerably (see figure 6 and the following tables) the more advanced genus, *Pernettya,* must have been derived from a very primitive species of *Gaultheria.*

Tribe VIII ANDROMEDEAE Drude
Figs. 3, 6

Flowers perfect, normally pentamerous. Stamens usually twice the number of corolla lobes. Anthers awned or awnless, opening by terminal pores. Ovary 5-celled (rarely 4-6-celled) with numerous ovules in each cavity. Fruit a loculicidal, usually depressed, 5-celled (rarely 4-6-celled)capsule. Wood diffuse porous. Vessel angular in outline with thin, even walls. Vessel perforation plates scalariform, never porous. Vessel side wall pits elongate-elliptic opposite to round opposite. Tertiary thickening of vessel wal's present. Medullary rays Heterogeneous Type I.

Andromeda, Leucothoe and *Chamaedaphne* form a group of genera with morphological characteristics so similar that taxoonomic interchange of species between the genera has been common. All three are deciduous or evergreen shrubs or trees with alternate leaves. Ovary structure and capsular structure among the genera differ in but insignificant details. The 8 to 12 (normally

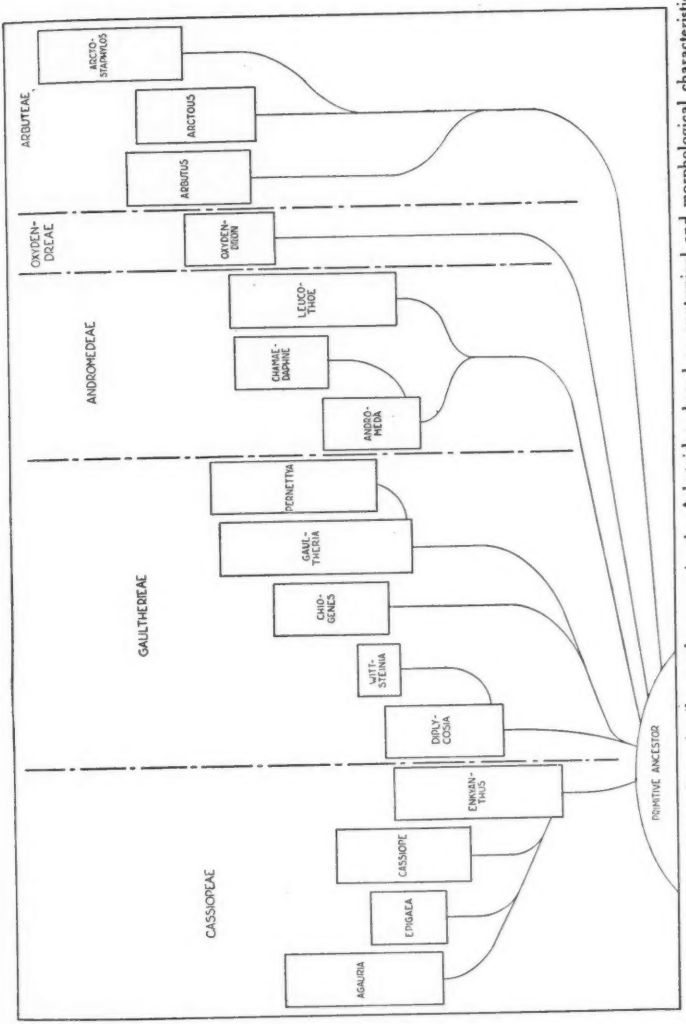

Fig. 6. Phylogenetic relationship of tribes and genera in the Arbutoideae based on anatomical and morphological characteristics.

GAULTHERIA L.

Tabulation of anatomical characteristics varying between the species.

G. acuminata Ch. & Schl. (NY—Schultes & Blas 155); *G. costaricensis* (Donn. Sm.) Small (NY—Pittier 3032); *G. shallon* Pursh. (NC—Adams 52, Adams 321); *G. procumbens* L. (NC—herbarium sheet 3910); *G. tenuifolia* (Phil.) Sleumer (NY—Ljuigner 698); *G. ramossissima* Benth. (NY—Killip & Small 17273); *G. lancifolia* Small (NY—Balls 4845); *G. perplexa* T. Kirk. (NY—Meebold 4353); *G. antipoda* Forst. (NY—Anderson 208).

	G. acuminata	*G. costaricensis*	*G. shallon*	*G. procumbens*	*G. tenuifolia*	*G. ramossissima*	*G. lancifolia*	*G. perplexa*	*G. antipoda*
Vessel diameter									
Maximum	44μ	47μ	44μ	33μ	39μ	33μ	29μ	33μ	34μ
Minimum	18μ	20μ	20μ	13μ	16μ	13μ	16μ	10μ	13μ
Average	30.6μ	30.1μ	27.7μ	27.2μ	23.3μ	22.5μ	22.3μ	21.0μ	22.2μ
Vessel length									
Maximum	849μ	694μ	596μ	687μ	559μ	580μ	510μ	439μ	496μ
Minimum	212μ	198μ	212μ	189μ	170μ	149μ	135μ	192μ	99μ
Average	579.0μ	475.6μ	430.0μ	425.2μ	366.7μ	359.6μ	340.4μ	321.3μ	312.1μ
Ratio	1:19	1:16	1:16	1:17	1:16	1:16	1:15	1:15	1:14
Vessel perforation									
% scalariform 9+ bars	100	80	94	90	94	100	87	16	66
% scalariform 8— bars	0	20	6	10	6	0	13	84	34
% porous	0	0	0	0	0	0	0	0	0
Average no. of bars	14.7	11.8	14.1	11.3	13.6	17.2	11.4	6.3	11.1

10) stamens are always included. Generic distinction is made primarily upon characteristics (of perhaps debatable consistency) of anther, corolla and calyx structure, and upon the method by which flowers are borne. It is certain that these three genera are very closely related.

It thus is not surprising that they do not radically differ from each other in anatomical characteristics. As figure 3 will show there is virtually complete consistency in a majority of critical anatomical criteria. Only in vessel dimensional data and in vessel perforation structure are there demonstrable differences between the genera.

Mainly for the purpose of simplification, the genus *Andromeda* in this study includes species that have at one time or another been assigned to the genera *Xolisma, Lyonia* and *Pieris* as well as *Andromeda*. As can be seen from the following table the species studied differ among themselves in no important anatomical respects. They are, therefore, anatomically at least, as closely related as species of many other taxonomically well established genera. Treated as a genus, the specific variation within the genus is not as great as, for instance, *Enkyanthus, Agauria, Gaultheria* or several others.

Andromeda is without doubt the primitive genus of the tribe as evidenced by its higher average number of bars in the scalariform perforations, and by the greater average length of its vessels.

PERNETTYA Gaud.

Tabulation of anatomical characteristics varying between the species.

P. mucronata Gaud. (NY—Cordini 73); *P. ciliata* (Cham. & Schl.) Small (NY—Balls B. 4794); *P. mexicana* Camp (NY—Camp 2604); *P. Pentlandii* DC. var. *parvifolia* (Benth.) Weld. (NY—Pennell & Hazen 9823); *P. lanceolata* (Hook. f.) Bull. & Hill (NY—Givlin & Long no number); *P. buxifolia* Mart. & Gal. (NY—Balls B. 4187).

	P. mucronata	*P. ciliata*	*P. mexicana*	*P. Pentlandii*	*P. lanceolata*	*P. buxifolia*
Vessel diameter						
Maximum	36μ	33μ	26μ	34μ	42μ	31μ
Minimum	16μ	13μ	10μ	16μ	16μ	11μ
Average	25.7μ	24.1μ	18.2μ	23.8μ	29.3μ	23.1μ
Vessel length						
Maximum	672μ	616μ	630μ	658μ	538μ	545μ
Minimum	142μ	170μ	135μ	142μ	156μ	149μ
Average	433.2μ	405.5μ	381.5μ	368.8μ	337.6μ	333.3μ
Ratio	1:17	1:17	1:21	1:15	1:12	1:14
Vessel perforation						
% scalariform 9+ bars	100	100	80	80	36	89
% scalariform 8— bars	0	0	20	20	56	11
% porous	0	0	0	0	8	0
Average no. of bars ...	15.8	17.7	12.4	11.1	8.0	12.7

ANDROMEDA L.

Tabulation of anatomical characteristics varying between the species.

A. nitida = *Pieris nitida* (Bartr.) B&H. (NY—Morong no number); *A. myrsinifolia* = *Xolisma myrsinifolia* (A. Rich.) Small (NY—Howard 6221); *A. cubensis* = *Pieris cubensis* (Griseb.) Small (NY—Shafer & Leon 13662); *A. obtusa* = *Xolisma obtusa* (Griseb.) Small (NY—Shafer 4115).

	A. nitida	*A. myrsinifolia*	*A. cubensis*	*A. obtusa*
Vessel diameter				
Maximum	37μ	50μ	39μ	44μ
Minimum	15μ	20μ	13μ	13μ
Average	24.1μ	32.2μ	26.2μ	27.0μ
Vessel length				
Maximum	566μ	708μ	623μ	545μ
Minimum	219μ	234μ	120μ	170μ
Average	411.2μ	477.0μ	392.9μ	278.6μ
Ratio	1:17	1:15	1:15	1:14
Perforation plate				
% scalariform 9+ bars	100	100	100	100
% scalariform 8— bars	0	0	0	0
% porous	0	0	0	0
Average no. of bars	24.0	18.5	20.7	19.4

Chamaedaphne is sometimes considered a subgenus of *Lyonia*. This conception appears erroneous since those species studied that have been classified in *Lyonia* (*Pieris nitida* and *Pieris cubensis*) resemble other species of *Andromeda* more than they resemble *Chamaedaphne*. This monotypic genus (*Chamaedaphne calyculata* (L.) Moench.; NY—Grassl 2435) shows advances over *Andromeda* in reduction in average vessel length and in number of bars comprising the scalariform vessel perforations. Because of the close parallel in anatomical and morphological characteristics *Chamaedaphne* is thought to have arisen directly from *Andromeda* (figure 6).

The third closely related genus, *Leucothoe*, occupies a position between *Andromeda* and *Chamaedaphne* based on anatomical characteristics (figures 3 and 6). As a genus its anatomical variation (see following table) overlaps both genera, reaching not so primitive a level as *Andromeda* nor one so advanced as *Chamaedaphne*.

The fact that the most primitive species of both *Leucothoe* and *Andromeda* are at about the same level of advancement indicates that neither gave rise directly to the other. *Leucothoe* doubtless arose from an ancestor common to *Andromeda* (figure 6) and the many similarities of structure between the two genera indicate that the point of divergence must have been quite recent. Thus the present day Andromedeae as here constituted stand as a particularly closely related, well-knit generic group.

LEUCOTHOE Don

Tabulation of anatomical characteristics varying between the species.

L. Catesbaei Gray (NY—Ruth 6625); *L. cordifolia* Meissn. (NY—Glaziou no number); *L. organensis* Gardn. (NY—Glaziou 19170); *L. hispidula* Meissn. (NY—Regnele 186); *L. ambigua* Meissn. (NY—Gardner 4987); *L. mexicana* (Hemsl.) Small (NY—Hinton 3899).

	L. Catesbaei	*L. cordifolia*	*L. organensis*	*L. hispidula*	*L. ambigua*	*L. mexicana*
Vessel diameter						
Maximum	33μ	37μ	39μ	41μ	40μ	54μ
Minimum	13μ	11μ	11μ	15μ	13μ	18μ
Average	22.3μ	26.0μ	23.6μ	25.2μ	24.4μ	33.6μ
Vessel length						
Maximum	736μ	616μ	644μ	552μ	538μ	481μ
Minimum	283μ	191μ	177μ	212μ	120μ	163μ
Average	499.7μ	426.8μ	382.2μ	361.6μ	347.5μ	327.0μ
Ratio	1:22	1:16	1:16	1:14	1:14	1:10
Vessel perforation						
% scalariform 9+ bars	100	100	86	100	100	71
% scalariform 8— bars	0	0	14	0	0	29
% porous	0	0	0	0	0	0
Average no. of bars ..	15.9	21.9	13.7	18.4	17.2	9.7

Tribe IX **Oxydendreae** Tribus nova

Figs. 4, 6

Corolla with 5 short lobes. Calyx persistent. Stamens 10. Anthers awnless, opening by terminal chinks. Disk 10-lobed. Ovary 5-celled with numerous ovules. Capsule loculicidally 5-valved. Wood diffuse porous. Vessels angular in outline with thin, even walls. Vessel perforations scalariform to porous. Vessel side wall pits scalariform, elongate-elliptic opposite and round-opposite. Tertiary thickening of vessel walls present. Medullary rays Heterogeneous Type IIB.

This tribe includes only the monotypic genus *Oxydendron* (*Oxydendron arboreum* DC.; NC—herbarium sheet 5238) so the tribal characteristics are naturally those of the genus. The combination of characteristics such as the dry, loculicidal capsule, the high percentage of porous vessel perforations (figure 4), the presence of tertiary thickening of vessel walls and Heterogeneous Type IIB medullary rays is more than sufficient to warrant erection of a separate tribe for this genus. It is hardly possible that *Oxydendron* could have evolved from any other present day genus of the family. A comparison of *Oxydendron* with its apparent close relatives, the Andromedeae for example, points up the virtual impossibility of such great advances in wood anatomy having been made without consequent advances having been made in floral and capsular structure as well, even at a greatly retarded rate of development. Such has not occurred and it must be concluded that *Oxydendron* is the survivor of a comparatively long evolutionary sequence paralleling that of the other tribes. In this phylogenetic sequence anatomical development

proceeded at a faster rate than morphological development. This has resulted in *Oxydendron's* having attained a real level of advancement considerably higher than its supposed relatives (figure 6), a level of advancement obscured by a consideration of morphological characteristics alone.

Tribe X ARBUTEAE Drude

Figs. 5, 6

Corolla of 4-5 fused petals, deciduous. Anthers awned, opening by terminal pores. Ovary of 4-10 cells. Fruit a many-seeded berry or berry-like fruit with 4-10 one-seeded nutlets (sometimes variously fused in 2's and 3's, or coalesced into a single sclid stone). Wood diffuse porous to semi-ring porous. Vessels angular in outline with thin and even to slightly uneven walls. Vessel perforation scalariform with few bars to porous. Vessel side wall pits elongate-elliptic opposite and round opposite. Tertiary thickening of vessel walls present. Medullary rays Heterogeneous Type IIA, extending uninterruptedly from the wood into the bark.

This tribe, composed of *Arbutus, Arctostaphylos* and *Arctous,* was originally established upon characteristics of calyx, anther and fruit structure. When anatomical data is considered along with these morphological characters it becomes even more apparent that the Arbuteae is a natural group (figure 5). The combination of Heterogeneous Type IIA medullary rays, presence of abundant vessel wall tertiary thickening, and absence of many-barred scalariform vessel perforations clearly sets these three genera apart from the other tribes of the Arbutoideae. The odd extensions of the medullary rays from the wood into the tissues of the bark is likewise an anatomical peculiarity of this tribe.

The Arbuteae appear to be considerably more advanced than other tribes in this subfamily. This is evidenced not only by type of fruit but by the tendency to form semi-ring porous wood, the comparatively short average vessel element length, the relatively low vessel diameter to length ratio, the reduced average number of bars in the scalariform perforations and other characteristics of wood anatomy. All three genera are quite closely related and differ primarily in ovary and fruit structure.

Monotypic *Arctous* (*Arctous alpina* Niedz.; NY—Ferguson no number) is probably least advanced of the three genera. It is the only genus that has not yet produced porous vessel perforation plates. Although retaining thin, even vessel walls it has acquired semi-ring porous wood and its vessel diameter to length ratio is lowest in the tribe (figure 5). *Arctous* shows a rather close relationship to *Arctostaphylos* in its berry-like fruit with variously separable one-seeded nutlets.

Arbutus, with some 20 or more species, is perhaps a slightly more advanced genus even though its wood is characteristically diffuse porous and its vessel diameter to length ratio is higher than *Arctous* (figure 5). This conclusion is based upon the large number of porous vessel perforation plates.

Considerable anatomical variation occurs among the species of *Arbutus* as may be determined from the following table:

ARBUTUS L.

Tabulation of anatomical characteristics varying between the species

A. xalapensis HBK (NY—Lyonnet 646); *A. Arizonica* (A. Gray) Sargent (NY—"herb. T. S. Brandegee"); *A. Menziesii* Prsh. (NC—herbarium sheet 788, Parry; 789, Grant); *A. texana* Buel. (NY—C. H. & M. T. Mueller 1031); *A. Donnell-Smithii* Small (NY—Standley 82362).

	A. xalapensis	*A. Arizonica*	*A. Menziesii*	*A. texana*	*A. Donnell-Smithii*
Vessel diameter					
Maximum	47μ	46μ	36μ	46μ	36μ
Minimum	13μ	15μ	13μ	13μ	13μ
Average	27.1μ	28.0μ	26.2μ	25.4μ	23.5μ
Vessel length					
Maximum	496μ	587μ	375μ	340μ	439μ
Minimum	142μ	163μ	99μ	127μ	99μ
Average	365.2μ	258.3μ	227.2μ	218.7μ	183.4μ
Ratio	1:13	1:9	1:9	1:9	1:8
Vessel perforation					
% scalariform 9+ bars	0	0	0	0	0
% scalariform 8— bars	44	22	68	0	48
% porous ..	56	78	32	100	52
Average no. of bars ..	2.9	1.7	2.8	–	2.5

Arbutus is distinct among the genera of the Arbuteae because of its fleshy, many-seeded fruits.

Arctostaphylos occupies the most advanced position in the tribe inasmuch as it has developed semi-ring porous wood, shows a tendency toward unevenly thickened vessel walls and has virtually achieved completely porous vessel perforations (figure 5). Its 4-10 seeded fruit must be considered more advanced than the many-seeded berry of *Arbutus*. This genus, like *Arbutus*, shows great variation in anatomical characteristics among its species.

As in the Andromedeae the many similar characteristics of these genera indicates a common origin for the tribe. Two developmental lines probably arose from this common ancestor, one giving rise to *Arbutus* and the other culminating in *Arctous* and *Arctostaphylos* (figure 6).

The development of *Arbutus* was characterized by retention of many features of the ancestor, such as the many-seeded fruit, diffuse porous wood and thin, even vessel walls. Principal advancement was made in evolution of a high percentage of porous perforations.

The other line of development was characterized by development of few-seeded fruits and semi-ring porous wood. A divergence of this phylogenetic line resulted in establishment of *Arctous* with no porous perforations and a low vessel diameter to length ratio, and *Arctostaphylos* with practically com-

plete porosity with a higher vessel diameter to length ratio and incomplete development of uneven vessel walls. Upon this consideration is based the view that neither could have arisen from the other. It is more likely that each arose from a recent but extinct ancestor and subsequently each has accelerated development of specific anatomical characters.

ARCTOSTAPHYLOS Adans.

Tabulation of anatomical characteristics varying between the species

A. crustacea East. (NC—Adams 80); *A. pajaroensis* Adams (NC—Adams 883); *A. nissenana* Merriam (NC—Adams 841); *A. andersonii* var. *auriculata* Jepson NC—Adams 676); *A. viscida* Parry (NC—Adams 619).

	A. crustacea	*A. pajaroensis*	*A. nissenana*	*A. andersonii*	*A. viscida*
Vessel diameter					
Maximum	49μ	39μ	52μ	47μ	36μ
Minimum	15μ	16μ	16μ	16μ	15μ
Average	27.5μ	26.5μ	29.8μ	25.2μ	23.1μ
Vessel length					
Maximum	488μ	481μ	474μ	552μ	382μ
Minimum	198μ	156μ	149μ	163μ	163μ
Average	363.8μ	310.0μ	312.8μ	281.7μ	254.1μ
Ratio	1:13	1:12	1:11	1:11	1:11
Vessel perforation					
% scalariform 9+ bars	0	0	0	0	0
% scalariform 8— bars	8	14	0	0	10
% porous ..	92	86	100	100	90
Average no. of bars ..	1.7	2.4	–	–	2.1

DISCUSSION OF THE SUBFAMILY

It has been apparent throughout the foregoing discussion that each of the tribal groups of genera in the Arbutoideae is monophyletic in origin. It is also apparent that all genera of the subfamily are rather closely related. It follows, therefore, that this entire group probably originated from a single ancestor or ancestral group. Such an ancestor, existent or presently nonexistent, can be reconstructed with reasonable accuracy. In order to give rise to the tribal groups we now know this ancestor could not have been more advanced in any morphological or anatomical characteristic than is now evident in any genus. Consequently this ancestor probably fulfilled the stipulations of the following list of generic characteristics: 1) flower pentamerous at least; 2) calyx of separate sepals free from the ovary; 3) corolla sympetalous; 4) ovary superior, of at least 5 cells; 5) ovules numerous in each cavity of the ovary; 6) fruit a dry, loculicidal, many-seeded capsule with at least 5 cells; 7) wood diffuse porous; 8) vessels angular in outline with thin, even walls; 9) vessel end wall angle considerably less than 30°; 10) vessel perforation

plates scalariform averaging more than 35 bars; 11) vessel side wall and wood parenchyma pits scalariform; 12) vessel wall tertiary thickening absent; 13) vessel element average length greater than 678μ; 14) vessel diameter to length ratio greater than 1:28; 15) medullary rays Heterogeneous Type I.

From this reconstructed ancestor arose five distinct developmental lines (figure 6) each characterized by retention of certain primitive features, slow evolution of other features, and rapid development of still others. Thus the Cassiopeae still retain the primitive capsular fruit, pentamery in floral structure, diffuse porous wood, angular vessels with thin, even walls, absence of vessel wall tertiary thickening and the primitive type medullary ray. Their advancement over their primitive ancestor is evidenced in reduction in the number of bars comprising the vessel perforation plates, in formation of some porous perforations, in development of elongate-elliptic opposite and round opposite vessel side wall pits from scalariform pits (some of which remain), in gradual shortening of average vessel element length, and in decrease in vessel diameter to length ratio.

Progress in the Gaultherieae affected most noticeably the character of the fruit. A gradual, step-by-step transition from dry capsule to juicy berry is shown in the existent genera of the tribe. With this has come a tendency to achieve tetramery of floral structure from the more primitive pentamerous condition. For the first time in the family thick and uneven vessel walls are found and for the first time in the subfamily vessel wall tertiary thickening is found, each derived condition being typical not of the entire tribe but of only a portion of it. Average vessel element length and vessel diameter to length ratio are both reduced. Again a tendency to develop elongate-elliptic and round vessel side wall pits is noted. In some cases the primitive scalariform pits have disappeared altogether.

Many primitive features remain characteristic of the Gaultherieae, however. Wood type is still diffuse porous. Vessels of some genera still have thin, even walls with no tertiary thickening. Vessel perforation plates are typically scalariform in this tribe. Although the average number of bars has been greatly reduced this tribe as yet has not developed porous perforations to an appreciable or significant extent. Medullary rays are still of the primitive structure.

The Andromedeae are more advanced than the lower members of the Cassiopeae and Gaultherieae but not as advanced as the higher genera of those tribes (figure 6). A compact, closely related group, the Andromedeae can be traced easily to their primitive ancestor by their capsular fruit and essentially pentamerous flowers. Likewise they have diffuse porous wood, angular vessels with thin, even walls, vessel perforation plates that are scalariform only, and their medullary rays are of Heterogeneous Type I.

Phylogenetic advancement of the Andromedeae involved shortening of the average vessel element length and reduction of diameter to length ratio, reduction in number of bars making up the scalariform perforations, development of elongate-elliptic and round vessel side wall pits with consequent loss of the scalariform type, and, significantly, the formation of relatively heavy tertiary thickening of the vessel walls. The Andromedeae is the first tribe of the subfamily yet discussed, all of whose members exhibit this advanced characteristic.

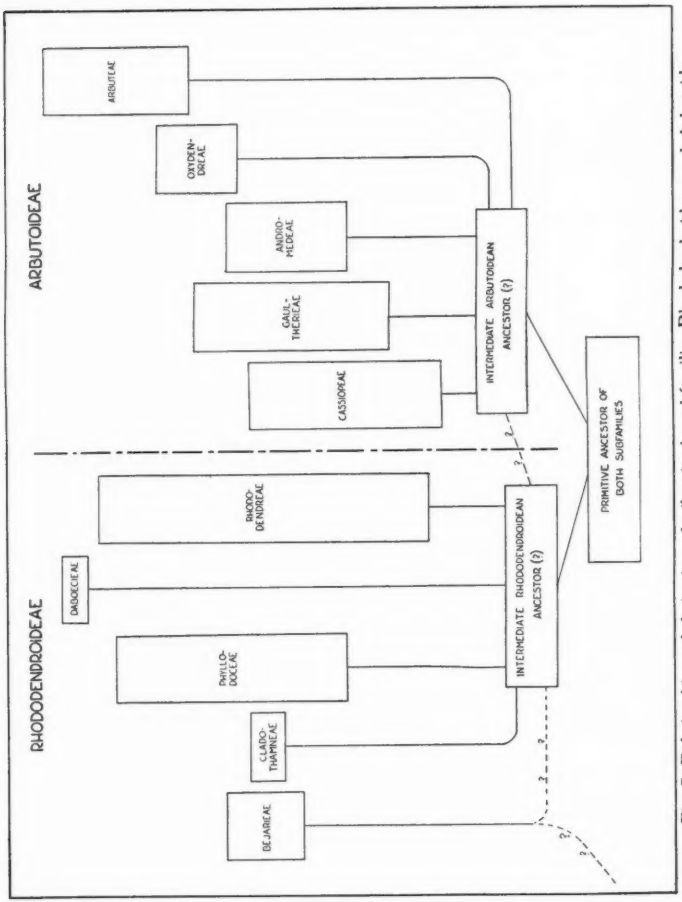

Fig. 7. Relationship and derivation of tribes in the subfamilies Rhododendroideae and Arbutoideae

As previously discussed the Oxydendreae is represented in our present day flora by a single genus and species. However it appears certain that *Oxydendron arboreum* is but the culmination of a phylogenetic line longer than that of any other tribe except the Arbuteae (figure 6). This species retains primitive characteristics of floral and fruit structure, wood type and vessel wall structure. Nevertheless considerable advancement over its primitive ancestor has been attained with reference to the presence of vessel wall tertiary thickening, shortening of average vessel element length and reduction of vessel diameter to length ratio. Elongate-elliptic and round opposite vessel side wall pits have been developed, although, as in the Cassiopeae and the Gaultherieae to some extent, this progression has not extended to the point where scalariform pits have been completely lost.

The high percentage of porous vessel perforations and the Heterogeneous Type IIB medullary rays are factors that establish *Oxydendron* as a comparatively highly evolved genus. The latter characteristic alone virtually precludes the possibility of very close relationship to any of the preceding tribes. *Oxydendron* appears to be the sole survivor of probably a lengthy phylogenetic sequence marked by accelerated development in vessel perforation plate and medullary ray structure.

Like *Oxydendron* the genera of the Arbuteae appear to be remnants of a long developmental line (figure 6). Tetramery has become fairly well established. None of the members of this tribe still produces a capsular type fruit. Transition from diffuse porous to semi-ring porous wood is being accomplished and vessel wall tertiary thickening is well developed. One genus has acquired unevenly thickened vessel walls. Vessel element length and diameter to length ratio have been greatly reduced. Tertiary thickening of the vessel walls is well developed and scalariform side wall pits have been lost. Vessel perforation structure has progressed to the stage where all are now either few-barred scalariform or porous. Finally, the primitive Heterogeneous Type I medullary rays have developed into Heterogeneous Type IIA. All in all, a comparatively high degree of specialization has been reached by the Arbuteae. Many intervening forms representing the step-by-step progression from the subfamily's common ancestor evidently have been lost and this tribe's three members stand as the truncated ends of a long phylogenetic line with three quite recent branches.

Fig. 7. Relationship and derivation of tribes in the subfamilies Rhododendroideae and Arbutoideae

CONCLUDING REMARKS

Evidence has been presented indicating a monophyletic origin for the five tribes of the Arbutoideae and a hypothetical ancestor has been reconstructed. It will be recalled that in the first paper of this series a similarly reconstructed ancestor was proposed for all tribes of the Rhododendroideae with the exception of the Bejarieae. This ancestor was pictured as having been a shrub having a regular, polypetalous, 7- or 8-merous flower with a superior ovary of at least 20 cells. Its wood would have been diffuse porous with Heterogeneous Type I medullary rays. Vessels would have been angular, thin and even-walled, quite long and slender, with a diameter to length ratio of at least 1:24, devoid of tertiary thickening. Many-barred scalariform perforations of the

highly inclined vessel end walls would have been characteristic as would scalariform vessel side wall pits.

By comparison this projected ancestor differs in but few respects from that of the Arbutoideae. The fact that all genera in these two subfamilies have a great many similar anatomical and morphological characteristics suggests strongly that these two ancestors were actually the same or at most were closely related forms of the same group (figure 7). The characteristics enumerated for the supposed primitive Rhododendroidean ancestor would be equally applicable to a supposed Arbutoidean ancestor. The primitive ancestor reconstructed above in the discussion of this subfamily therefore actually would represent an intermediate form which gave rise directly to the presently existent Arbutoideae but which itself may have originated from the Rhododendroidean ancestor.

In this connection one should recognize also that both Rhododendroidean and Arbutoidean ancestors may have been intermediate forms and that actual phylogenetic divergence may well have occurred prior to development of either reconstructed ancestor (figure 7). In any case both subfamilies, with the exception of the Bejarieae, are evidently of monophyletic origin and the concept of broad evolutionary trends thought to have taken place in the development of the tribes is not affected.

The existent forms which are least advanced, based on an overall view of both anatomical and morphological evidence and are, therefore, closest to their primitive ancestors are species of *Rhododendron* and *Enkyanthus*. *Enkyanthus* ranges from the Himalayas to Japan. *Rhododendron* is virtually worldwide in distribution but is found in greatest concentration also in the Himalayas and southeastern Asia in general. It is logical to conclude that their primitive ancestor was also native to this area.

The present worldwide distribution of these subfamilies must be explained by great migrations of their genera or their extinct predecessors intermediate in morphological and anatomical complexity between the original primitive ancestor and the present day forms. It is probable that this primitive ancestor gave rise to the immediate ancestors of the nine demonstrable tribes quite early, and that these tribal ancestors then began migrations into new areas, successively setting off new forms. Some of these must have become extinct, some must have subsequently given rise to still newer forms, and some remain today as recognizable genera scarcely changed from the time that they first appeared.

An early mass migration generally northward from the area of origin must have been undergone by the tribal ancestors of all tribes so that their descendents, except as noted later, became established as components of the high northern, circumpolar flora. Migration then apparently spread eastward into North America and westward across Siberia into northern Europe. Thus the precursors of our present day Rhododendroidean and Arbutoidean genera became diffused throughout the circumpolar flora. Movement southward into localized areas such as the South Atlantic states or southwest Europe was made by immediate ancestors of the existent genera or by these genera themselves.

Many evidences of this "great circle" migration remain today. In six of the nine tribes (again excluding the Bejarieae) a study of the present distributional limits of genera progressing from primitive to advanced indicates that just such a migration has been made.

In the Cladothamneae *Tripetaleia* remains in Japan as a descendent of a more primitive form deposited there in the northward migration. *Cladothamnus* became established along the western Canadian and U. S. mountain ranges and *Elliottia* migrated southward from the circumpolar area into the southeastern Atlantic states.

In the Rhododendreae, *Rhododendron*, the most primitive genus, is concentrated in southeastern Asia but may be found to a lesser extent (with reference to number of species) throughout all the areas in which migration of this tribe is presumed to have occurred. *Rhododendron* gave rise to *Azalea* probably in eastern Asia. There *Menziesia* and *Azaleastrum* arose from *Azalea*. The Asiatic species of *Azalea* were apparently lost as *Azalea* migrated into and became established in North America. *Menziesia* still remains in eastern Asia although it too is typically North American. *Azaleastrum* is today found along the path followed by its ancestors—from China to Japan and along the North American Rocky Mountains.

A branch of this phylogenetic line resulted in the establishment of *Ledum* and *Tsusiophyllum*. *Ledum* is now circumpolar in distribution while *Tsusiophyllum* or an ancestor of it became established in Japan and remained there.

Migration of the Cassiopeae along the same route is shown by a comparison of the ranges of its present day genera. *Enkyanthus*, already shown to be the family's most primitive genus, is found from the southeastern Himalayas to Japan and Cochin China. From *Enkyanthus* was derived *Cassiope* whose range is from the Himalayas northward into Japan and Eastern Siberia, from there eastward across Canada into Greenland, extending southward into Oregon and the Rocky Mountains, and westward across Siberia into Scandinavia. From *Cassiope* originated *Epigaea*, at first probably circumpolar in extent, but whose two surviving species have become established separately in Japan and the eastern seaboard of North America. The isolated occurrence of *Agauria* in the mountains of central Africa, in Madagascar and in Mauritius is puzzling and cannot be explained completely by this hypothesis. A migration southward from the northern circumpolar region would have had to occur since anatomical evidence indicates *Agauria* arose from the ancestor of *Cassiope* and *Epigaea*, but there is little evidence that such migration took place.

In the Gaultherieae *Diplycosia* is the most primitive genus. It is found from Bhutan in the southeastern Himalayas southward into the Malay Peninsula. A recent ancestor of *Diplycosia* apparently gave rise to *Chiogenes* and then *Gaultheria*. *Chiogenes* may be found along its path of migration in Japan, in northern North America and from Newfoundland south to North Carolina. *Gaultheria*, which arose later, is largely North American and has moved along the eastern highlands and along the western mountain ranges into Central and South America.

However some species of *Gaultheria* still remain along the route from the Himalayas to Japan and the occurrence of *Gaultheria* in Australia, New

Zealand and Tasmania indicates that a southeastward migration from *Gaultheria's* point of origin along the Malay peninsula across the East Indies into the Australian group must have taken place. That this is probable can be determined by again considering *Diplycosia. Diplycosia* has spread from the southeastern Himalayas into the Malay peninsula. It seems quite certain that *Wittsteinia* originated from *Diplycosia* and *Wittsteinia* is native only to southeastern Australia.

The present distributional pattern of *Pernettya* also strengthens the above outlined two-pronged migratory path of *Gaultheria* and at the same time supports the view that *Pernettya* is derived from *Gaultheria.* Anatomical and morphological evidence leads to the conclusion that *Pernettya* originated from *Gaultheria. Pernettya's* range is discontinuous, being found in the Australian group mainly in Tasmania and New Zealand, and to a great extent in Central and South America. Significantly, these areas represent the ends of the migratory routes along which *Gaultheria* apparently moved.

The Gaultherieae therefore must have migrated in two directions, one northward followed by *Chiogenes, Gaultheria* and *Pernettya;* the other southeastward followed by *Diplycosia, Wittsteinia* and also *Gaultheria* and *Pernettya.*

The Andromedeae is largely North American but representatives of the most primitive genus, *Andromeda,* are yet found from the Himalayas northward into Japan and the circumpolar regions. Along the way *Chamaedaphne* arose from *Andromeda. Chamaedaphne* now is distributed in northern Europe, northern Asia and northern North America southward along the Alleghanies. *Leucothoe* developed from *Andromeda's* ancestor and is now mostly North American but has penetrated into Central and South America as far as Brazil.

As has been stated, the Arbuteae is a rather highly derived group and its intermediate ancestors linking it to the original subfamilial ancestor appear to have been lost. Representatives of *Arbutus, Arctous* and *Arctostaphylos* having circumpolar distribution however are still found, and *Arctous* remains essentially circumpolar. *Arbutus* has moved into the warm Mediterranean regions and along the west coast of North America into Mexico. *Arctostaphylos* has migrated southward into middle Europe, and in North America southward along the Atlantic coast to Pennsylvania and along the Pacific coast into Mexico.

Three tribes in the two subfamilies do not exhibit clear cut evidence of this migration. Two are monotypic and we can but surmise as to their origin and migration.

Daboecia is the most advanced genus of the Rhododendroideae and is thought to be the sole survivor of a long phylogenetic line. Since *Daboecia* is found in southwestern Europe along the Atlantic coast it is reasonable to assume that its present distribution so far removed from its presumed point of origin merely corroborates the evidence derived from a study of its anatomy and morphology. The great advance shown by *Daboecia* over its primitive ancestor is matched by the great distance *Daboecia* has moved from its area of origin. Hence intermediate forms between this ancestor and *Daboecia*

were developed as migration proceeded northward, then westward and finally southward into *Daboecia's* present range, but these intermediate forms are extinct or unknown.

The present distribution of *Oxydendron* undoubtedly may be explained in the same manner. *Oxydendron,* as has been pointed out, is obviously not very closely related to any present day Arbutoidean genus. Also it is rather more advanced than has hitherto been suspected. Its range from Pennsylvania along the Alleghanies to Florida is also as far removed from its area of origin as it is advanced anatomically over the subfamily's ancestor. Intervening forms showing both the migratory route northward, eastward and then southward from the Himalayas into *Oxydendron's* present range, as well as the demonstrated anatomical specialization that has occurred, have, as in the case of the Daboecieae, been lost or are as yet unknown.

The Phyllodoceae present a confusing picture with regard to its migration. Three generic groups originating from a common tribal ancestor comprise this tribe. The Phyllodocean ancestor arose from the common subfamilial ancestor which is thought to have existed in the southeastern Himalayas. The main tribal developmental line leading to *Bryanthus* gave rise to *Diplarche* or to a direct ancestor of it which remained in the Himalayan region. *Phyllodoce* was then developed and it is now of circumpolar distribution. From *Phyllodoce* arose closely related *Bryanthus* which is found today in eastern Siberia. Thus this generic group apparently underwent the same northward migration engaged in by all other tribes.

A second generic group is composed of *Rhodothamnus* and *Kalmia. Rhodothamnus* is found from Siberia southwestward into the European Alps. *Kalmia* is now restricted to eastern North America from Canada to Cuba. The ancestor common to these two genera must have been circumpolar, having reached this area by a northward migration from the Himalayas similar to that demonstrated for other tribes.

The third generic group leads from the tribal ancestor through *Ledothamnus* and *Leiophyllum* to *Loiseleuria.* In this progression considerable anatomical specialization took place and sympetaly was achieved as well as reduction in carpellary number. On the basis of anatomical and morphological evidence *Ledothamnus* gave rise to *Leiophyllum* and *Leiophyllum* gave rise to *Loiseleuria.* However *Ledothamnus* is endemic to the Guianas of South America, *Leiophyllum* is native to the Atlantic coast from New Jersey to Florida, and *Loiseleuria* is circumpolar in range. This would suggest a northward migration from South America for this generic group. Since this is contrary to the migratory paths evidently followed by all other tribes and generic groups the balance of evidence does not support this view. It is possible that *Leiophyllum* and *Ledothamnus* were once of circumpolar distribution and after migrating southward became established permanently only in the areas they now occupy. Likewise it is conceivable that a now extinct ancestor whose distributional range extended continuously from northern Canada into South America gave rise first to *Ledothamnus,* second to *Leiophyllum* and last to *Loiseleuria* in the areas they now inhabit. Neither of these hypotheses is satisfactory. As for the first, there is no evidence that *Ledothamnus* ever existed

very far beyond its present range. As for the second, anatomical and morphological evidence points strongly to the fact that *Ledothamnus* itself gave rise to *Leiophyllum* and *Leiophyllum* to *Loiseleuria* rather than that they are all derived from a common intermediate ancestor.

The Bejarieae has not been discussed from a distributional standpoint. As pointed out in the first paper of this series *Bejaria* was probably derived from an unknown ancestor entirely different from all other genera of the two subfamilies. Largely a South American genus extending but slightly into North America, *Bejaria* is far removed from any other genus of the Ericaceae from anatomical and distributional standpoints and no close relationships can be drawn.

The genera of the Rhododendroideae and Arbutoideae, with the exception of *Bejaria*, have all evidently been derived from a single, unknown, probably extinct ancestor.. This ancestor seems to have been native to southeastern Asia, the Himalayas in particular. From this ancestor have arisen nine more or less distinct tribal groups of present day genera which, in aggregate, are practically of worldwide distribution. In general the present tribal and generic distributional limits were established as a result of two great migrations. One of these may be traced northward from the area of origin almost to the Arctic. There migration took place both east and west across Asia, Europe and North America. Southward movements then occurred mainly along the western and eastern mountain ranges of North America and into Europe and the Mediterranean area. The second of these migrations took place along the Malay peninsula, the islands of the East Indies and into the Australian and New Zealand island group.

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Book Reviews

CHEMICAL INSECT ATTRACTANTS AND REPELLENTS. By Vincent G. Dethier. The Blakiston Company, Philadelphia, Pa., 1947. xv+289 pages, 69 figs. $5.00.

Not only entomologists but biologists in general will find much of interest in Dr. Dethier's book. He deserves real credit for the skill and clearness with which he has brought together and evaluated the information on this subject.

In the author's own words the book "is neither a compilation of recipes for attractant and repellent substances nor a manual on insect control" but "represents an attempt to bridge the borderline between chemoreception and the broader aspects of behavior based upon it."

The book consists of ten chapters. In chapter one, the history of the subject is presented; in chapters two to five, the various types of attractants are discussed; chapter six is devoted to olfactometers and threshold concentrations; chapter seven to baits and traps; and chapter eight to repellents. These eight chapters are based on factual material and indicate a distinct familiarity with the field. Chapter nine is on the chemical basis of taste and olfaction; and chapter ten on the evolution of feeding preferences. The subjects presented in these last two chapters require interpretations which are not always easy, and to further complicate matters, there are here many unknowns in our knowledge. In the reviewer's opinion, Dr. Dethier handles this subject matter in a distinguished manner.

As our science has developed the specialists know less and less of each others' special fields of knowledge. Books like this, if read, would do much to remedy this situation since they make conveniently available the widely scattered literature of the subject.

Dr. Charles T. Brues has written a "Foreword" to the volume. Each chapter has a bibliography, and the book concludes with author and subject indices.—LEE H. TOWNSEND, University of Kentucky, College of Agriculture and Home Economics, Lexington.

VIE ET MOEURS DES AMPHIBIENS. By F. Angel. Payot, 106 Boulevard Saint-Germain. Paris. 1947. 8vo., 317 pp., 138 figs. Paper cover, 360 francs (= $3.03, Jan., 1948).

Few really exhaustive studies on a world-wide basis of the natural history of amphibians exist. The author says that none exist in his own language, at least of recent date, and the statement could have been expanded to encompass all languages. The scope is, to be sure, more limited than Noble's in the latter's *Biology of the Amphibia*, but the treatment of natural history scarcely touches the vast field explored by Angel's book in considerable detail. Although written in French, the purely English-reading public of the United States will find the illustrations so useful and the basically close relation of French and English so obvious that the book should be regarded just as indispensable as though it were composed in a more familiar tongue.

Terrarists and others interested in the habits of amphibians, which play such a conspicuous role in terraria anywhere, will welcome the wealth of detail in natural history as an aid and stimulus to their own work and hobby. The appeal of the book is popular, by style and very content, but also scientific, based as it is upon actual fact gleaned from first-hand knowledge of one of the leading herpetologists of the world and from many writings of other scientists.

The information included is of a vast variety. Thirteen pages are devoted to a history of classification, zoogeography, and ancestry of modern amphibians. All of the remainder of the book is devoted to natural history. Two chapters (XIV and XV, pp. 264-294) concern only the habits of the amphibians of France, but the rest of the book draws upon material of world-wide range, with essentially equal emphasis upon all geographic regions. An extraordinarily careful search of the literature preceded the account, as indicated by the thoroughness of treatment and the careful citation of source of information. The partial bibliography of most important works is extremely useful.

Chapter I (27 pp.) treats the subject of "Ecology-ethology," and includes subheadings on (a) size, (b) a few habits and habitats (13 pp.)., (c) longevity, (d) hibernation and estivation, (e) altitudinal distribution, and (f) growth.

Chapter II (10 pp.) concerns "Locomotion and movements," and includes discussions of variation in locomotor organs as well as actual methods of movement.

Chapter III (21 pp.) treats "Nutrition and voice," with subheadings on (a) mouth and teeth, (b) tongue and its role in capture of food, (c) food, (d) enemies, (e) fasting and (f) voice. Although the discussion of types of vocal sacs is excellent the apparent oversight of Liu's summary is an important omission.

Chapter IV (18 pp.) discusses "Sense organs," with subtopics on (a) sensations, (b) taste, (c) smell, (d) touch (8 pp.), (e) hearing, (f) sight, and (g) light-sensitivity of the skin.

Chapter V (14 pp.) is entitled "Respiration," and the following sub-topics are included: (a) types of respiration, (b) gills, (c) lungs, (d) lung atrophy, and (e) integumentary respiration.

Chapter VI (21 pp.) treats "The skin and the coloration," under the following subheadings: (a) scales of the apoda; the lymphatics, (b) water conservation in amphibians, (c) dermis and epidermis, (d) coloration, (e) chromatophores, (f) melanism and albinism, and (g) types of patterns and mimicry.

Chapter VII (13 pp.) discusses "Glands and venoms," with subheadings on (a) mucus and granular venoms and glands, (b) actively and passively venomous animals, (c) venomous urodeles, (d) venomous anurans, (e) blood toxicity, and (f) natural immunity of amphibians to their own and other venoms.

Chapter VIII (17 pp.) concerns "Psychism and instincts," with sections on (a) definition, (b) influence of environment, (c) associative memory and acquisition of habits, (d) individual and specific protective instincts, (e) sexual behavior of *Alytes*, (f) protection of young in *Pipa*, and (g) temporary secondary instincts and the clasping reflex.

Chapter IX (15 pp.) treats "Vital resistance and regeneration." Subheads are: (a) temperature and animal heat, (b) resistance to desiccation, (c) reactions at different temperatures, (d) resistance to asphyxiation and starvation, and (e) regeneration of mutilated organs.

In Chapter X (35 pp.), the longest in the book, "Reproduction" is discussed under (a) generalities, (b) sexual dimorphism and hormones, (c) secondary sexual characters, (d) hermaphroditism, (e) spermatozoa, (f) fertilization and oviposition, and (g) hybridization.

Chapter XI (27 pp.) summarizes "The eggs," under (a) aspect of eggs, (b) oviposition, (c) number of eggs, (d) nests and site of oviposition, (e) parental transport of eggs, (f) viviparity and ovoviviparity, and (g) incubation and hatching.

Chapter XII (18 pp.) concerns "Larvae and tadpoles," including (a) phases of metamorphosis, (b) mouth and corneous teeth, (c) size of larvae, (d) duration of larval life and neoteny, and (e) parental care of larvae.

The economic aspect to man of amphibians is treated in Chapter XIII (13 pp.). In this chapter the name *Rana mugicus* is used, presumably for *Rana pipiens*, although the name itself is more appropriate for *Rana catesbeiana*, of which a suspiciously similar synonym already exists, *R. mugiens*. In either case I can find no precedent for use of the name *R. mugicus*, which can be considered (1) a *lapsus* for *R. mugiens*, (2) a *nomen nudum* or (3) synonym of *R. pipiens* or *R. catesbeiana*. The figure labelled *Rana pipiens* (fig. 123) is obviously of *R. catesbeiana*.

Chapters XIV and XV concern only the amphibians of France, as mentioned previously, and the last chapter (XVI, 8 pp.) discusses "Certain legends, errors and prejudices."

Except for the absence of an index, the book is admirably organized. Here and there one may lament the lack of further detail, but for the space utilized the choice of material could scarcely be improved upon. The work shall certainly prove to be an indispensable compendium and thought-provoking digest for the teacher, the amateur and the professional alike.—HOBART M. SMITH, University of Illinois, Urbana.

ANIMAL BIOLOGY. By Michael F. Guyer. Harper & Brothers, New York. 1948. Fourth edition, 784 pp., 433 text figs. $4.50.

To the many users of M. F. Guyer's *Animal Biology* and others, the fourth edition of this text will be welcomed, especially because it comes nearer to the goal of welding the fundamentals of morphology and physiology into a comprehensible sum than previous editions. This text will also be welcomed by teachers in medical schools, for all too often students of medicine enter upon their professional work with inadequate appreciation of the vast interrelationships that exist between structure and function. Guyer's text on *Animal Biology* in its fourth edition will help the pre-medical student to strike a happy and useful balance between anatomy on the one hand and the physiological functioning of anatomical parts on the other. This will be a useful text too for the general student not intending to pursue a medical curriculum.

There has been little change in the arrangement of parts over the old third edition. In the first section the student is introduced to the world of life, the manifest characteristics of living things, the substance of living things, classification of animal forms, interpretation of plants and animals and ecological relationships. There then follows a section on structure. Here the author presents a descriptive analysis of the frog as a representative animal following which lesser forms such as *Euglena, Paramoecium, Hydra, Obelia, Lumbricus* and *Cambarus* are considered.

The third part of the book is devoted to the organ systems and their functions. Germ cells, fertilization, and heredity mechanisms are considered in the fourth section. The origin of the various kinds of animals constitutes the substance of the fifth part of the text where the evidences for evolution from morphology, embryology, physiology, geographic distribution, and paleontology are examined together with various theories on how evolution has come about. The final and sixth section of the textual material deals with a synoptic survey of the animal kingdom. Following this are sections devoted to reference books, glossary, visual aids and the index.

There are two new features of this fourth edition which should enhance the book's usefulness to the student. The first of these is a Chapter Review which follows at the end of each chapter except in Part VI. In this the student is first charged to "understand what is meant by all terms shown in *bold-faced* type" and secondly is given a series of questions to answer. The second of these two new features is a section on visual aids. This gives a list of motion picture films and filmstrips suitable for classroom showing to illustrate various biological phenomena, together with the addresses of producers or distributors from whom they can be obtained. This list of visual training aids should encourage the use of such materials in the classroom. Its inclusion in this edition is timely in view of the greater emphasis now on such teaching facilities as a result of the vast experiment in mass education of the last war.

There have been several changes or additions to the body of the text. Of minor importance is a shift in the sequence of the chapters on organ systems. The skeletal and integumentary systems are considered firstly as opposed to the chapter on digestive and respiratory sytsems in the older edition. This shift probably makes for a more logical sequence. Of major importance is the expanded treatment of the material on protoplasm and the cell. Considerable detail with reference to cellular physiology has been incorporated into a new chapter on "The Cell in More Detail." Basic inorganic and organic constituents of the cell are outlined together with the intricacies into which these fit in cellular metabolism. The student is introduced to enzymes and electrical aspects of protoplasmic change. Hydrogen-ion concentration ordinarily reserved for introductory chemistry courses is presented very clearly. Following this comes a description of buffer systems and maintenance of acid-base balances of the body. Permeability of and passage of stuffs through cell membranes are also explained in lucid manner.

Another important addition to the text is the inclusion of a discussion on the Rh factor in human blood.

The chapter on digestive systems has been improved by a rather detailed analysis of the vitamins and their role in health and disease. The vitamin B complex and vitamin

K are well treated. Dicumoral recently shown to be the agent producing sweet clover disease in cattle and as a pharmaceutical to prevent blood clotting, is mentioned in connection with the anti-hemorrhagic vitamin.

Five figures of the third edition have been eliminated and eighteen new ones have been inserted. Several of the latter are old figures redrawn for greater effectiveness, and one is a color print of an old black and white. Generally, the figures in this text leave much to be desired. New, clear and attractive illustrations and photographs would add considerably to the desirability of this book as a classroom text.

On page 681 the sequence of events in the life cycle of the human malarial organisms is described in part as follows: "In man, the spindle-shaped *sporozoites* injected in the saliva of the mosquito when it bites, invade the red blood corpuscles (Fig. 401, D, 1-7) and become schizonts. . . ." This view is no longer held as valid by parasitologists since work of the past twelve years has shown that these organisms probably develop in the reticulo-endothelial system or the parenchyma cells of the liver before becoming schizogonic forms in circulating blood. Fig. 401 on page 680, therefore, should be eliminated or revised according to present day views.

On page 387 the trematode sporocyst and redia are given as examples of larval forms which reproduce paedogenetically. Some specialists would not agree with this interpretation, for sporocysts and redial stages in trematode life cycles are regarded as adult organisms rather than larval forms. Certain of the monogenetic trematodes would have been a wiser choice as an example of paedogenetic development.

It seems to this reviewer that an unnecessary amount of space and separation of subject material are involved in the presentation of two synoptic reviews of the animal kingdom, both in reduced form in the early pages of this book, and again in more expanded form in Part VI at the end of the text. Considerable usefulness of these synoptic treatments is eliminated by separating the diagnostic features of the phyla (front of book) from the main (back of book) synopsis. It is thoroughly confusing to the novice.

The group of the Acanthocephala is listed in the synopsis in Part VI of the book but is ignored in the front section synopsis. In placing the Acanthocephala among the phyla the author states that this group of helminths is "often regarded as a class in the phylum Nemathelminthes, but there is nothing to warrant such a view." Yet in spite of this statement and evidence which he cites for the cestode affinities of the group, the author places the Acanthocephala in such a position to leave no doubt of their association with the round worms. The Acanthocephala should be regarded as a phylum related to the Platyhelminthes.

Without exhaustive analysis, the index to this new fourth edition seems not to have received the same careful treatment as the body of the text. At least twenty omissions of importance and two page references for items already included have been noted.

There seem to be few typographical or printers' errors in this work. The book is attractively bound, but the quality is not on a par with the previous edition. The paper may be lighter in weight although it is difficult to judge by comparison with a well-thumbed and hard-used third edition which the reviewer has used in several classes of students.—DAVID R. LINCICOME, Department of Medical Microbiology, University of Wisconsin Medical School, Madison.